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MYCOLOGIA

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VOL. LIII MARCH–APRIL, 1961 NO. 2

JULIAN HOWELL MILLER (1890–1961)

W. A. CAMPBELL

(WITH PORTRAIT)

Julian Howell Miller, son of the late Charles and Annie Miller, was born in Washington, D. C. on 16 July, 1890 and died at Athens, Georgia on 25 March, 1961. Dr. Miller at the time of his retirement in 1958 was Head of the Department of Plant Pathology and Plant Breeding and Regents Professor of Botany at the University of Georgia where he served the University and the state for over 37 years.

His boyhood was spent at Quitman, Georgia and the major portion of his adult life at Athens. Here he married the former Miss Mary Douglas Morris who died in 1957. He is survived by a daughter, Miss Anne L. Miller and a son, Julian H. Miller, Jr., both of Athens.

Dr. Miller received his B.S.A. in 1911 and his M.S. in 1924 from the University of Georgia, and his Ph.D. from Cornell University in 1928. His academic honors included membership in Phi Beta Kappa, Phi Kappa Phi, and Sigma Xi. He served as president of the following professional organizations: American Mycological Society; Southern Section, American Phytopathological Society; Southern Section, American Botanical Society and the Georgia Academy of Science. He was also a member of the British Mycological Society, and the New York Academy of Sciences. His name appears on over 100 publications dealing with plant pathology and mycology.

With the exception of several years of military service with the A.E.F. in World War I, Dr. Miller's entire life was devoted to teaching

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and research. He acquired a world-wide reputation as a mycologist and was recognized as the leading authority on the Xylariaceae. A comprehensive monograph of the genus *Hypoxylon* had just gone to press at the time of his death. Monographic treatments of other genera of the Xylariaceae were also in the advance stages of preparation. Dr. Miller's unfinished work on the Xylariaceae will be published as a bulletin by the University of Georgia.

JULIAN HOWELL MILLER

The foregoing list of events and accomplishments give little indication of the true measure of the man or his place in the minds and hearts of his contemporaries. To me and to hundreds of others, students, fellow teachers, and research workers, townspeople, horticulturalists, agriculturalists, and plain Georgia citizens, Julian Miller exemplified all the virtues of the true southern gentleman. His unfailing patience, courtesy,

and scholarly attainments made an indelible impression upon his students. He inspired many to make plant pathology and mycology their careers. His record as a productive research worker, despite the pressure of teaching and administrative duties and often the lack of adequate facilities, demonstrates that the true research spirit cannot be denied. He will long be remembered by many as a great scholar, teacher, researcher, and friend.

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STUDIES ON THE INTRACELLULAR AMINO ACIDS OF PENICILLIUM ROQUEFORTI

E. MEYERS [1, 2] AND S. G. KNIGHT

(WITH 2 FIGURES)

Penicillium roqueforti Thom can readily utilize ammonium sulfate as the sole source of nitrogen for the synthesis of amino acids and subsequently proteins (Meyers and Knight, **1958**). Various workers have shown that microorganisms are capable of "pooling" amino acids, the pool serving as a reservoir of building blocks on the direct pathway to proteins from ammonia and the carbon skeleton. The existence of intracellular amino acid pools in filamentous fungi has been reported (Fuerst and Wagner, **1957**; Pillai and Srinivasan, **1956**; Simonart and Chow, **1953**), but little quantitative work has been done. The purpose of this investigation was to analyze the amino acid pool in both vegetative hyphae and in spores of *Penicillium roqueforti*, and to determine the size of this pool in hyphae under various experimental conditions.

METHODS

Penicillium roqueforti, Minnesota strain P 108-2, was used throughout this study. The maintenance of the stock culture and the preparation and standardization of the spore inoculum have been described (Meyers and Knight, **1958**).

The synthetic medium and the conditions of incubation for submerged growth have been described (Meyers and Knight, **1958**). To obtain a good crop of spores, urea was substituted for ammonium sulfate in the synthetic growth medium. A solution of urea was sterilized by passage through a Seitz filter and added aseptically to provide 2 g nitrogen per liter. In several experiments 2 mg each of adenine, guanine, xanthine, thymine, cytosine and uracil were sterilized in 100 ml of the medium.

The boiling water method (Spiegelman, Halvorson, and Ben-Ishai, **1955**) was used to obtain the amino acids from the pool. A 10% sus-

[1] Present address: The Squibb Institute for Medical Research, New Brunswick, New Jersey, U. S. A. This work was supported in part by a grant from the National Science Foundation (N.S.F. G-3359).

[2] Part of a thesis submitted to the Graduate School of the University of Wisconsin in 1958, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

pension (W/V) of dried cells in distilled water was placed in a vigorously boiling water bath for 30 minutes, the cellular debris was removed by centrifugation and discarded and the supernate was saved for analysis. Radioactive cells were fractionated according to the method of Roberts et al. (**1955**). The cold trichloroacetic acid (TCA) soluble fraction was extracted 3 to 5 times with an equal volume of ether to remove the TCA.

Conventional semi-micro digestion and Nesslerization techniques were used to determine total nitrogen. Soluble protein was measured according to the biuret method of Gornall, Bardawill and David (**1949**). Amino nitrogen was assayed by the quantitative ninhydrin method (Moore and Stein, **1954**). The results of these determinations are expressed in terms of leucine equivalents. Arginine was separated from other pool components by ion exchange chromatography (Winters and Kunin, **1949**) and quantitated by the Sakaguchi method of Gilboe and Williams (**1956**). The components of the pool were analyzed by the buffered paper chromatography technique of McFarren (**1951**). Specific color tests were used also to detect certain amino acids. The specific tests were applied after the test solution had been resolved by paper chromatography. Arginine was detected by the method of Jepsen and Smith (**1953**), tyrosine by the technique of Acher and Crocker (**1952**), and tryptophan by the method of Smith (**1953**); proline was sought for by the isatin method (Smith, **1953**) and hydroxyproline by the method of Jepsen and Smith (**1953**).

Radioactive samples were plated in stainless steel planchets and counted at infinite thinness.

RESULTS

Penicillium roqueforti contains an amino acid pool, the size of which ranges from approximately 5 to 15% of the total nitrogen of the vegetative cells. Alanine was present in the highest concentration with glutamic acid and aspartic acid in slightly lower concentrations. Isoleucine, leucine, arginine and serine were present in still lower concentrations, while glycine, threonine, lysine, phenylalanine, tryptophan, tyrosine, histidine, methionine and valine were present in trace amounts. All of these amino acids were found also in a protein hydrolysate of the mycelium; proline and hydroxyproline were not detected in either the amino acid pool or in the protein hydrolysate. The qualitative pattern of the pool was quite stable. No major changes were detected over a period of seven days incubation in the synthetic growth medium. The size of the pool, however, in terms of the percentage soluble nitrogen, decreased

TABLE I

PER CENT NITROGEN IN THE AMINO ACID POOL DURING SUBMERGED GROWTH IN A COMPLETE SYNTHETIC MEDIUM

Culture age (days)	Dry weight mycelium/flask (mgs)	Pool nitrogen (% of total cell N)
3	472	11.5
4	640	7.6
5	660	—*
6	655	6.8
7	657	6.1
8	654	8.6
9	675	5.2

* Sample lost.

during a nine-day incubation period (TABLE I). This decrease may be a reflection of the accumulation of structural material rather than an absolute decrease in the percentage soluble nitrogen. The arginine content of the pool closely followed the variation of the total nitrogen in the pool (FIG. 1).

A similar study was made using spores and no qualitative differences were noted in the composition of vegetative cells and spores. In spores, too, alanine was judged to be the major component, followed by glutamic and aspartic acids.

Washed vegetative cells of *P. roqueforti,* 4 days old (i.e., in the middle of the rapid growth phase, Meyers and Knight, **1958**) were suspended in a nitrogen-free medium (the synthetic medium from which the ammonium sulfate was omitted) and harvested after 24, 48 and 72 hours of incubation. The pool levels were assayed by determining the percentage of total cellular nitrogen in the amino acid pool. TABLE II shows that the percentage soluble nitrogen of the nitrogen-starved cultures was higher than the comparable control. Similar observations in regard to total nitrogen have been made with yeast and bacteria under conditions of nitrogen deficiency (DeLey, **1955**). However, inclusion

TABLE II

EFFECT OF NITROGEN STARVATION ON THE LEVEL OF THE AMINO ACID POOL

Exp.	Incubation (hrs)	Pool nitrogen (% of total cell N)	
		Nitrogen-free medium	Nitrogen-containing medium
1	24	13.3	12.3
	48	7.8	6.9
	72	8.5	5.3
2	40	9.8	4.4

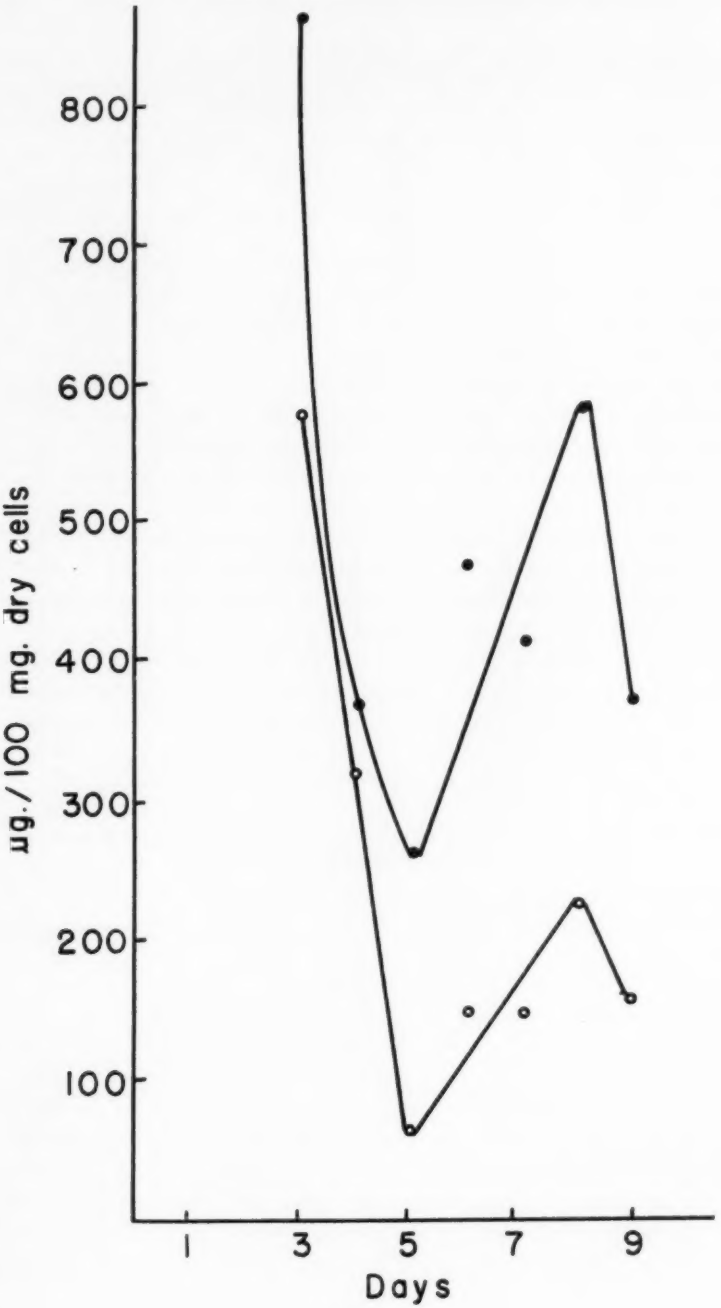

FIG. 1.

of purine and pyrimidine bases in an otherwise nitrogen-free medium greatly decreased pool levels (TABLE III). Washed, 4-day-old hyphae were depleted markedly of their amino acid content and paper chromatograms showed that this depletion involved all the amino acids; several amino acids were reduced to such a level that they did not form the blue compound with ninhydrin on paper. In all instances, the size and intensity of the spot were less from the pools of cells supplemented with the bases than from the nonsupplemented cells. In subsequent experiments similar results were obtained, though the magnitude of the response varied. To rule out the possibility of leakage into the medium, cells labelled with an isotopic amino acid (2^{14}C glycine or uniformly labelled ^{14}C arginine) were suspended in a nitrogen-containing and a nitrogen-free medium for periods up to 48 hours. No radioactivity could be detected in either medium. It was concluded, therefore, that the

TABLE III

EFFECT OF PURINE AND PYRIMIDINE BASES ON THE SIZE OF THE AMINO ACID POOL

Supplement	Pool nitrogen (% of total N)	Pool arginine (μM/100 mg dry cells)	Leucine equivalents per ml pool
With $(NH_4)_2SO_4$ but no bases*	4.4	0.54	7.40
With $(NH_4)_2SO_4$ and with bases	1.7	0.03	1.53
No $(NH_4)_2SO_4$ but with bases	3.7	0.07	3.22
No $(NH_4)_2SO_4$ and no bases	9.8	0.51	12.32

* Base solution: adenine, guanine, xanthine, thymine, cytosine and uracil in a final concentration of 2 mg each in 100 ml medium.

presence of nucleic acid bases led to the depletion of the pool, probably by enhanced protein synthesis. That uptake of pool amino acids occurred when cells were suspended in a nitrogen-free medium is shown in FIG. 2. It is evident that, as glycine disappeared from the amino acid pool (cold TCA soluble fraction), it appeared in the nucleic acid (hot TCA soluble), protein and cell wall (hot TCA insoluble) fractions.

Preliminary experiments with inhibitors showed that 1×10^{-1} M sodium azide, 2×10^{-2} M sodium arsenate, and 5×10^{-4} M 2,4-dinitrophenol caused decreases in pool levels of washed and blended, 4-day-old mycelia even in the presence of exogenous ammonium sulfate. Diethylmalonate at 2×10^{-3} M did not decrease pool levels but inhibited the incorporation of 2^{14}C glycine into the cold TCA fraction, hot TCA soluble fraction, and hot TCA insoluble residue to the extent of about 50% in 11 hours.

FIG. 1. Fluctuation of arginine and total nitrogen of the amino acid pool during the course of incubation. ●, total pool N; ○, pool arginine.

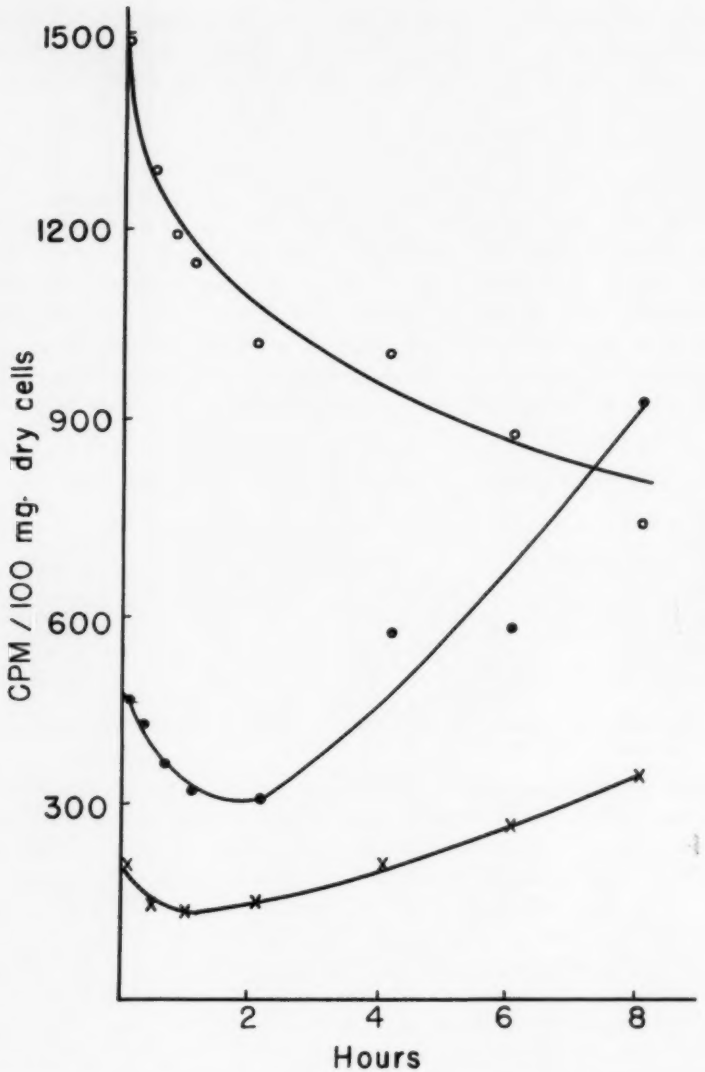

FIG. 2. Disappearance of radioactivity from the cold TCA soluble fraction and transfer into other fractions. The cells were incubated in a synthetic medium containing 20 μC of 2-^{14}C glycine, 5.7 μC per mg, for 7 min at room temperature, washed and resuspended in a nitrogen-free medium, and sampled at various time intervals. ○, Cold TCA soluble; ●, hot TCA insoluble; ×, hot TCA soluble.

DISCUSSION

The amino acid pool of *P. roqueforti* is relatively large, both qualitatively and quantitatively. The pool comprises all the amino acids found in the protein make-up of the hyphae, and about 5 to 15% of the total cellular nitrogen. This magnitude is similar to that of yeasts (Spiegelman et al., **1955**). The pool size decreases with time under conditions of normal growth (TABLE I); however, the type of growth of a filamentous fungus must be borne in mind. As a hypha grows, proportionately more structural material is formed and therefore the ratio of hot water-soluble to hot water-insoluble nitrogen in the sample decreases. This may account for the decrease in percentage soluble nitrogen.

The pool level increased during nitrogen starvation, a finding which can be interpreted as a type of internal replenishment, probably by the breakdown of labile protein(s) to replenish the amino acid pool. Halvorson (**1958**) and Mandelstam (**1958a, b**) reported protein breakdown under conditions of nitrogen starvation in *Escherichia coli*. Depletion of the pool is accomplished readily, however, by the inclusion of purine and pyrimidine bases in the medium that lacks nitrogen. The presence of these bases might favor the synthetic reaction to such an extent that synthesis far exceeds breakdown, or the rate of breakdown might be considerably lessened. Either event would lead to a diminished pool.

The filamentous growth and continuity of protoplasm in the filamentous fungi suggest the interesting possibility of protein turnover or the "reuse" of amino acids by movement from older nongrowing cells up to younger cells or even hyphal tips.

SUMMARY

Penicillium roqueforti was found to contain an intracellular amino acid pool comprising approximately 5 to 15% of the total cell nitrogen. The pool contained most of the common amino acids; however, proline and hydroxyproline were not detected. The qualitative pattern of the pool was relatively stable and was similar in both vegetative cells and spores. The pool level of vegetative cells increased upon incubation in a nitrogen-free medium and decreased when incubated with several inhibitors of respiration, or purine and pyrimidine bases. Indications were obtained of an internal pool replenishment mechanism in nitrogen-depleted cells.

DEPARTMENT OF BACTERIOLOGY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN

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STUDIES ON CALVATIA GIGANTEA. I. GERMINATION OF THE BASIDIOSPORES [1]

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(WITH 4 FIGURES)

The importance of the discovery of new tumor-retarding agents of potential value in the inhibition of human malignant growths has been emphasized in recent years through the rapid increase in laboratory and clinical testing of oncostatic substances. The discovery of such a substance(s) in *Calvatia gigantea* (Pers.) Lloyd developed from a study of higher fungi initiated by the late Dr. Eugene H. Lucas. Synonyms for *Calvatia gigantea* are: *Lycoperdon giganteum* Persoon, *Bovista gigantea* Nees, *Lycoperdon Bovista* Fries, *Calvatia maxima* Morgan, *Calvatia Bovista* MacBride, *Calvatia primitiva* Lloyd.

As a possible method for obtaining strains which might produce a greater yield of the oncostatic principle(s), germination of *C. gigantea* spores was attempted.

Stevens (**1957**) reported on environmental factors influencing *in vitro* growth of basidiomycetes and their elaboration of biologically active substances. A portion of his study included *C. gigantea*.

Sedlmayr (**1960**) studied the nutritional requirements of *C. gigantea* and other *Calvatia* spp., with particular emphasis on vitamins. She demonstrated that each of the investigated strains was totally deficient for thiamine.

Reports of attempts to germinate basidiospores of *C. gigantea* are relatively absent from the literature. Hoffman (**1859**) appears to have been the first investigator to report germination experiments on *Lycoperdon* spp. He reported that he germinated spores of *L. constellatum* Fr., *L. gemmatum* Batsch, and *Bovista plumbea* Pers. using a hanging drop technique. His paper mentioned the possibility that the germinat-

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ing spores in actuality were those of a *Penicillium* sp. However, the following year (**1860a**, **1860b**) his attempts to repeat these results failed. Failure in germination studies on *Bovista, Lycoperdon* and *Scleroderma* species was reported by Hesse (**1876**).

As of **1887**, De Bary believed that the basidiospores of most Gasteromycetes, including the Lycoperdaceae, had not been germinated. Brefeld (**1877**, **1908**) held a similar opinion.

Ferguson (**1902**) failed to germinate spores of *Calvatia cyathiformis* (Bosc.) Morgan, *Lycoperdon pyriforme* (Schaeff.) Fr., *L. gemmatum* and *L. wrightii* B. & C. Cool (**1912**) reported negative results for the attempted spore germination of *Lycoperdon* spp., *Bovista nigrescens* Pers. and *Scleroderma vulgare* Fr.

Since Swartz (**1928**) believed the exceptionally thick spore wall of the *Lycoperdon* spp. could hinder germination, he employed a wetting and drying technique in an attempt to make the wall more permeable. He was not able to germinate spores of *L. gemmatum, L. atropurpureum* Vitt., and *Bovista pila* B. & C. However, he stated he may have seen early stages of germination in a few spores of *Calvatia saccata* (Fr.) Lloyd, but was unable to repeat these observations. He claimed in this brief report to have had a limited success with *Lycoperdon pyriforme* (Schaeff.) Fr. Although he did not discuss his techniques, he reported cytological evidence which indicated a monokaryotic nuclear condition in the young germ tubes. Kaufman (**1934**), however, was unsuccessful when he employed several methods including Swartz's in attempts to germinate spores of *Bovistella radicata* (Mont.) Pat. and *Calvatia cyathiformis.*

Fries (**1941**) reported that he had succeeded in germinating spores of *Lycoperdon echinatum* Pers., *L. nigrescens* Pers., *L. pratense* Pers., *L. pyriforme, L. umbrinum* Pers., *Scleroderma aurantium* Pers. and several species of *Tricholoma* and *Boletus.* He mixed spores with melted malt extract agar before pouring plates, or seeded the surface of this agar. He then inoculated "Hefe X" (Yeast "X," i.e., unidentified) at 8–10 places on the surface of the malt extract agar. The incubation time varied from one species to another; 3 to 30 days was the time generally required for the appearance of colonies. The spores which were placed on the agar surface germinated earlier than those seeded into the agar. *Torulopsis sanguinea* (Schimon) Cif. & Red. activated germination nearly as well as the "Hefe X."

Fries postulated that the action of this yeast may be to neutralize inhibitors or to act as a germination stimulator. Fries' method gave less than 0.1% germination. Attempts to replace the yeast with several

vitamins were not successful. A later publication (**1943**) discussed his experimental methods in greater detail.

Kneebone (**1950**) summarized the successful germination studies on spores of the Gasteromycetes.

MATERIALS AND METHODS

The fall of the year is the best season for collection of *C. gigantea* sporophores in Michigan. Since these investigations were started in the spring and summer (**1957**) fresh material was not available. However, for the past 5 years, *C. gigantea* sporophore collections had been made by the late E. H. Lucas (Horticulture Department, Michigan State University), and stored at approximately 26–28° C. Sections from some of these sporophores (939A to 998A) were removed and kept in culture dishes (75 × 100 mm) at 26° C. But it soon became evident that these quantities were insufficient, and that this method increased the possibility of contamination. Thus, in the fall of 1957 and ensuing years, sporophores were collected (1096A to 1405B), kept intact or divided into 2 or 3 parts, and stored at room temperature (av. 26° C) affixed A, 10° C affixed B, and −18° C affixed C. Sporophores that had a relatively intact peridium were most desirable since these were generally less contaminated. Material was collected or sent from Michigan, several bordering states, Alaska, Canada and South America. It was found desirable to dry out moist puffballs for a few days at room temperature before placing them in storage.

When immature sporophores were collected, a mycelial transfer was cultured in a test tube containing medium A (see below). These sporophores were maintained at room temperature with the hope that they would produce basidiospores. In some cases this occurred.

Numerous media were used in attempts to germinate the spores of *C. gigantea*. The media considered to be of greatest interest are described. Other substrates are discussed more briefly in Results.

1. *Medium A Broth (Agar)* after Stevens (**1957**): The following constituents were added and made up to one liter of distilled water: 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 1.0 g KH_2PO_4, 0.01 g $FeSO_4 \cdot 7H_2O$, 15.0 g glucose, 15.0 g sucrose, 5.0 g Bacto-peptone, 5.0 g Bacto-yeast extract. pH 5.6. Fifteen g of Bacto-agar was added to made medium A agar when desired.

2. *Barley Extract:* Forty g of ground California bleached barley was added to one liter of distilled water, steamed for 45 minutes, filtered through cheesecloth and then autoclaved.

3. *Soil Extract:* Soil was collected from an area where the previous year a sporophore of *C. gigantea* had been found growing. Three hundred ml of this soil was added to one liter of distilled water and steamed for 45 minutes, then allowed to settle for one hour. The supernatant was clarified by suction filtration.

4. *Puffball Extract:* One hundred g of an immature *C. gigantea* sporophore was placed in one liter of distilled water and blended for 2 minutes in a Waring Blendor. Solid materials were removed by suction filtration.

5. *Malt Extract Agar:* Twenty-five g of Difco malt extract and 20 g of Bacto-agar were added to one liter distilled water.

6. *Sabouraud's Broth plus Yeast:* Difco Sabouraud's broth was prepared and 75 ml dispensed into 250 ml Erlenmeyer flasks. All flasks were inoculated with *Rhodotorula mucilaginosa* (Jörgensen) Harrison var. *sanguinea* (Schimon) Lodder (R. M. yeast).

7. *Medium A Broth plus Pancreatin:* A 2.5% solution of Pancreatin was filtered through a Seitz filter pad. One hundred ml of this was dispensed into 900 ml of medium A broth which had previously been autoclaved.

8. *Fries' (1941) Techiqnue, Modified* (malt extract agar plus R. M. yeast): Thirty ml of malt extract agar was placed in 75 ml Pyrex test tubes. After autoclaving, these tubes were placed in a 42° C water bath. When the medium equilibrated to 42° C, one ml of puffball spore suspension was dispensed into a tube of medium, shaken and poured into a Petri dish. Twenty-four hours later the R. M. yeast was inoculated on the surface of the medium at four equidistant spots, which were generally 1¼ inch apart and formed the corners of a hypothetical square. All plates were sealed with masking tape to prevent the medium from drying out. Unless otherwise stated, these were incubated at 26° C. Colonies which appeared under the cultures of R. M. yeast were not recorded. Since this yeast generally occupied 20% of the surface area of the medium, the number of colonies counted was at least 20% low.

Chloromycetin was added (previous to autoclaving) to all broths at a concentration of 100 mg/liter and 6 mg/liter for malt extract agar, unless otherwise stated.

Stock solutions were prepared for all vitamins. Aliquots were removed to attain desired concentrations. In all cases, vitamins were dispensed into media previous to autoclaving. All glassware was acid-washed for 3 days and then rinsed in distilled water.

Two types of shakers were used: (a) a New Brunswick Gyrotory shaker, operated at slow speed; (b) reciprocal shaking machine having

65 two-inch excursions per minute. Both shakers were kept in a 26° C (±1° C) constant temperature room.

All puffball cultures were maintained on medium A agar in unslanted tubes. Sufficient growth to cover the medium usually resulted after 2–3 weeks. At such time 5 ml of sterile Bayol oil (Penola Co.) was pipetted into these test tubes. This oil completely covered the medium and mycelium. The cultures were kept at room temperature.

Stock cultures of *Rhodotorula mucilaginosa* var. *sanguinea* (R. M. yeast) were maintained on Sabouraud's agar or malt extract agar slants at 12° C. This organism is considered to be the *T. sanguinea* used by Fries (**1941, 1943**). Kneebone (**1950**) held such an opinion. This organism was obtained from the American Type Culture Collection (NRRL strain Y-174).

To obtain suspensions of spores for germination studies a portion of the peridium of a sporophore was surface sterilized with 70% ethyl alcohol. This sterilized layer was removed and with tweezers, spore material was discarded to a depth of 1 cm. From the inner portion of this hole, less than 1 cm^3 of spore material was removed and placed in a sterile (semi-micro, monel metal) Waring Blendor. Two hundred ml of sterile distilled water was then added to the Blendor. A uniform spore suspension was obtained after blending for 10 seconds. The spore concentration was determined using an AO Spencer haemacytometer. Fresh spore suspensions were prepared for every experiment.

In germination experiments using broth, 75 ml of media was poured into 250 ml Erlenmeyer flasks and autoclaved. From the inner portion of the sporophore approximately 1 cm^3 of spore material was removed and dropped directly into each flask.

RESULTS

Since there appear to be no published reports of attempts to germinate the basidiospores of *C. gigantea*, our first experiments consisted of using various media at different temperatures, and pretreatment of spores.

In the first series of experiments various media were incubated at temperatures of 28° and 35° C. Chloromycetin was not used in these media. Experiments were terminated after 30 days incubation. This period was selected because Fries (**1941**) noted that 30 days was the longest period required for colonies to appear from *Lycoperdon* spp. These media were inoculated by lightly shaking a small portion of sporophore material above the plates.

Contamination by fungi and particularly bacteria was a great problem in these experiments, but a sufficient number of clean plates was obtained to evaluate the media. None of these media supported germination of basidiospores (TABLE I).

In further germination experiments, spores of *C. gigantea* were subjected to various pretreatments. After each treatment the spores were suspended in sterile distilled water for 24 hours and then examined microscopically for germination. Treated spores were also streaked on medium A, without chloromycetin, contained in Petri dishes. These

TABLE I

AGAR MEDIA USED UNSUCCESSFULLY IN PRELIMINARY ATTEMPTS TO GERMINATE BASIDIOSPORES OF CALVATIA GIGANTEA.[a] PLATES WERE INCUBATED FOR 30 DAYS AT 28° AND 35° C

Media	Sporophore number
1. Steamed puffball extract	948A, 961A, 970A, 971A, 983A
2. Blended puffball extract	948A, 961A, 970A, 971A, 983A
3. Soil extract	954A, 961A, 983A, 986A, 989A, 998A
4. Sabouraud's inoculated at 4 places with R. M. yeast	984A, 985A, 989A
5. Malt extract inoculated at 4 places with R. M. yeast (Fries, 1941)	984A, 985A, 989A, 995A
6. Soil extract inoculated at 4 places with R. M. yeast	995A, 984A
7. Steamed puffball extract inoculated at 4 places with R. M. yeast	995A
8. Potato dextrose	984A, 985A, 995A
9. Litman's oxgall	984A, 995A
10. Difco blood agar base	995A

[a] Spores were dispersed onto surface of media.

dishes were incubated for 20 days and examined periodically for germination.

Owing to contaminants it was necessary to use as many as 20 plates of medium A agar for each sample of pretreated spores. Pretreatments of spores are recorded.

1. Spores from sporophore #995A were washed with 95% ethanol for periods of 1, 5 and 10 minutes.
2. Spores from sporophore #989A were heated for 5 minutes at the following temperatures: 35°, 40°, 50°, and 60° C.
3. Spores from sporophores #972A and #986A were heated at 50°, 60°, 70°, and 80° C for intervals of 5, 10, 15, 20 and 25 minutes.

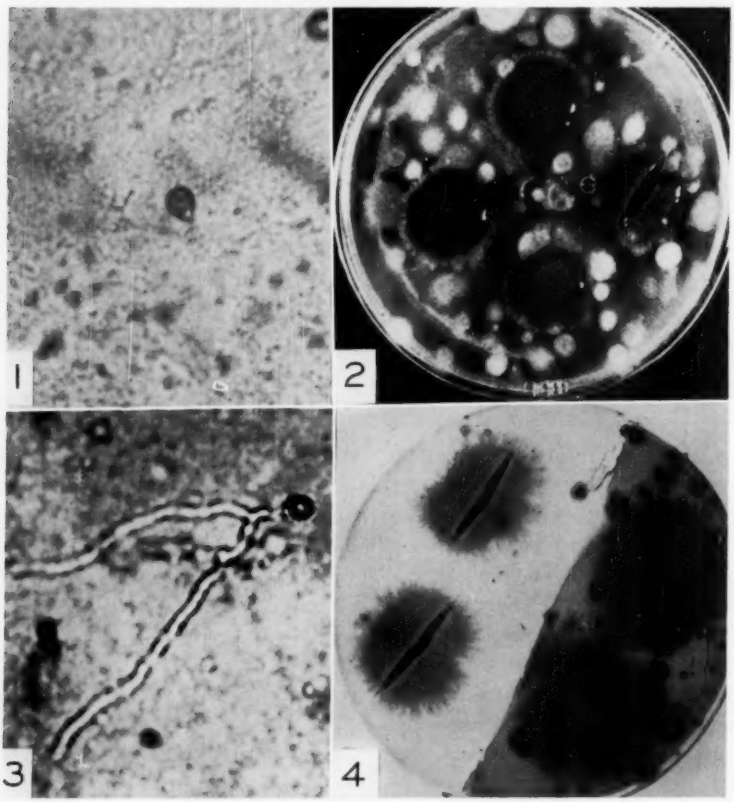
4. Spores from sporophores #995A and #986A were dipped into the following concentrations of sulphuric acid for 5 minutes: 1, 10, 25 and 50%.
5. The walls of spores from sporophore #1373A were etched using Nossal disintegrator.
6. Spores from sporophore #984A were incubated for 20 hours in sterile solutions of bile salts ranging from 0.25 to 15%.
7. Spores from sporophore #939A were alternately frozen and thawed six times over a three day period.
8. Spores from sporophore #1373A were placed in the following glucose concentrations for 24 hours: 0.5, 1.0, 5 and 10%.
9. Forty g of a *C. gigantea* basidiocarp (immature) was blended for 5 minutes with 500 ml of distilled water. This preparation was filtered through a Seitz apparatus. Spores from sporophore #1373A were incubated for 3 days in this preparation.
10. Spores from sporophore #1380A were incubated at 28° C for 24 hours in a 1% solution of Pancreatin extract that had been filtered through a Seitz apparatus.

None of these treatments were successful in germinating the spores of *C. gigantea*.

Barley extract broth inoculated with spores of *C. gigantea* #995A was used to study germination in liquid cultures. All flasks were incubated on a rotary shaking apparatus. During the first two weeks of incubation several of the flasks contained fungal growths which were identified later as contaminants. The remaining flasks were left on the shaker for several more weeks. Finally, after 9 weeks incubation, mycelium growing out of the original piece of spore inoculum was observed in three of the six remaining flasks. Samples of this mycelium were transferred to medium A agar for colony development. Since there is apparently no fungus generally classified as a laboratory contaminant that requires nine weeks from spore germination to colony development, this fungus was compared with a culture obtained from the basidiocarp. Both cultures were buff-brown in color. The centers were slightly raised and growth was relatively flat. The cultures turned the medium slightly brown and three weeks were required for these cultures to reach one inch in diameter. The hyphae were 2–3.5 μ in diameter and lacked reproductive structures except for occasional chlamydospores.

In subsequent experiments, using spores of *C. gigantea* #995A in barley extract broth, germination was observed microscopically after

7–8 weeks incubation (FIG. 1). The number of germinating spores calculated after eight weeks incubation indicated that less than one spore out of 20 million had germinated. An occasional spore appeared to have an appressorium. However, these structures were never observed giving rise to hyphae. Advanced stages of spore germination were never seen



FIGS. 1–4. 1. A germinated basidiospore of *Calvatia gigantea* #995A incubated on shaker for 8 weeks at 26° C in barley extract broth, × 950. 2. Colonies resulting from the germinated spores of *C. gigantea* in malt extract agar incubated at 26° C for 9 weeks. The 4 large, dark colonies are the R. M. yeast. 3. Germinated spore of *C. gigantea* incubated at 26° C for 6 weeks in malt extract agar inoculated with the R. M. yeast, × 950. 4. Colonies resulting from germinated spores of *C. gigantea* #1390AF incubated at 26° C for 8 weeks. Spores on the right side of the Petri dish were covered with malt extract agar while spores on the left side were not covered. The 4 larger colonies are the R. M. yeast.

when observed microscopically; as soon as the germ tube protruded to a length of approximately five μ, it appeared to be broken off by the action of the shaking machine.

Capillitium was never seen giving rise to hyphae; in fact, most of these threads did not contain complete cells.

From the above evidence it was decided that spores of *C. gigantea* had been germinated. Later, in support of this contention, mycelium derived in a similar manner demonstrated antifungal and tumor-retarding properties similar to those obtained using mycelium derived from *C. gigantea* basidiocarps (Bulmer and Beneke, in preparation).

It was of interest to determine if the spores of *C. gigantea* could be germinated in broths other than barley extract. Various broth media (TABLE II) were investigated. Spores from sporophore #995A were incubated for 90 days at 26° C. If no growth occurred after this period of time, it was considered that the spores would not germinate under the experimental conditions.

Spores from sporophore #995A were germinated in six of the ten media, the most successful of which were the barley and puffball extracts containing chloromycetin. Of the 19 flasks containing *C. gigantea* mycelium, 17 had chloromycetin incorporated into the broth. The media lacking the antibiotic were mostly contaminated with bacteria.

In later experiments, employing barley extract broth, spores were germinated from sporophores of *C. gigantea* #1096A, #1369A and #1389A. No germination was obtained using spores of *C. gigantea* #1370A, #1376A and #1380A.

These results indicated that some of the spores from certain sporophores of *C. gigantea* could be germinated.

Despite the fact that spores of *C. gigantea* had been germinated in broth, it was considered more desirable to germinate them in solid medium so that conditions necessary for germination could be more accurately defined and the chances of securing monosporous cultures might be increased.

Fries' (**1941**) technique which was successful in germinating spores of *Lycoperdon* spp. was modified slightly for these experiments. Spores of a naturally overwintered puffball (#1390A) were used. Forty Petri dishes containing malt extract agar (without chloromycetin) were seeded with spores and 20 of them were inoculated at 4 equidistant spots with R. M. yeast. No R. M. yeast was inoculated into the remaining plates. Each dish contained 36×10^6 spores and was incubated at 28° C.

Periodically, these plates were examined for evidences of spore germination. After 4 weeks, an average of 36 small white colonies (1 colony

per million spores inoculated) were seen growing in each Petri dish inoculated with the yeast. One hundred and eight colonies (3 colonies per million spores) were counted in each dish after 7 weeks incubation. Mycelium was transferred from several of these cultures and placed on slants of medium A agar. Two weeks later these cultures exhibited characteristics similar to the cultures which were derived from *C. gigantea* spore germination in barley extract broth. It was decided that these were colonies of *C. gigantea*. No colonies appeared in any of the plates

TABLE II

SPORE GERMINATION RESULTS FROM CALVATIA GIGANTEA #995A GROWN IN VARIOUS MEDIA

Broth	Chloromycetin incorporated	M.P.[a]
(1) Sabouraud's plus one day autoclaved R. M. yeast	yes	1/5
	no	0/5
(2) Sabouraud's plus three day autoclaved R. M. yeast	yes	0/5
	no	1/5
(3) Sabouraud's plus living R. M. yeast	yes	0/5
	no	0/5
(4) Autoclaved soil extract	yes	0/5
	no	0/5
(5) Seitz filtered soil extract	yes	0/5
	no	0/5
(6) Puffball extract, autoclaved	yes	3/5
	no	0/5
(7) Puffball extract, Seitz filtered	yes	3/5
	no	0/5
(8) Medium A	yes	0/10
	no	0/10
(9) Medium A plus pancreatin	yes	2/5
	no	1/5
(10) Barley extract	yes	8/10
	no	0/10

[a] Ratio of flasks containing mycelial pellets of *C. gigantea* to original number of flasks.

that had not been inoculated with the yeast. FIG. 2 shows this technique using spores from another sporophore.

In subsequent experiments, using spores from a minimum of eight sporophores, *C. gigantea* colonies appeared only in malt extract agar that had been inoculated with the R. M. yeast.

Owing to the fact that these colonies appeared to originate in the medium, it was not possible to microscopically observe if they were derived from *C. gigantea* spores. This difficulty was overcome by dispens-

ing 1 ml of a spore suspension containing 44×10^6 spores from #1390B evenly on the surface of malt extract agar in Petri dishes. One-half of this seeded surface was covered with an additional layer of malt extract agar which was cut from a previously poured Petri dish. Two dots of R. M. yeast were added to this surface and two dots to the uncovered surface. All plates were incubated at 28° C. On several occasions during the period between the 5th and 7th week of incubation the added section of medium from some of these dishes was removed and the germination of *C. gigantea* spores was observed microscopically (FIG. 3). The remaining dishes contained *C. gigantea* colonies after an additional 2 weeks incubation. This was considered evidence that spores of *C. gigantea* had been germinated in a solid medium.

It was observed in this experiment that the half of the plate which had the spores left uncovered had an average of 8 colonies, and the section that was covered had 65 colonies. The germination per million spores inoculated was 0.4 and 2.9, respectively. Throughout the ensuing months, the germination of spores from other sporophores verified the observation that a greater percentage of spores of *C. gigantea* germinates when covered with a layer of medium (FIG. 4). This rate was similar to that obtained when spores were seeded into the malt extract medium. In concurrent experiments when spores from #1390B were seeded into malt extract medium and inoculated with R. M. yeast, the germination rate was 2.8 per million. Since 2.9 spores per million germinated when dispensed on top of the medium and covered with additional agar medium cut from a previously poured Petri dish, it was concluded that seeding spores at 42° C did not affect germination results.

Fries (**1941**) attempted to replace the living yeast with various vitamins in his studies on *Lycoperdon* spp. spore germination. Although he failed, Cochrane (**1958**) mentioned that this co-culture with a yeast is suggestive of a vitamin effect.

The following vitamins were investigated. In every case Petri dishes containing malt extract agar seeded with *C. gigantea* spores and inoculated with R. M. yeast were a control. In these experiments spores from either 1402C or 1390B were used.

1. *Biotin:* Concentrations of 5, 10 and 15 μg/liter were attained in the following media: medium A lacking yeast extract, medium A lacking yeast extract and peptone, malt extract agar, and Magenmaltz agar (Fries, **1943**). In preliminary experiments, without chloromycetin, colonies which strongly resembled pure cultures of *C. gigantea* appeared in some of the above. These were subsequently always considered to be pure cultures of *C. gigantea* colonies for reasons outlined previously.

However, microscopic examination revealed there was a bacterium present. In subsequent biotin addition experiments, when chloromycetin was used to inhibit this bacterium, no colonies of *C. gigantea* appeared.

To investigate the effect of this bacterium further, 40 plates of malt extract agar (minus chloromycetin) were seeded with spores of *C. gigantea* #1400C. Ten of these plates (called control plates) were inoculated with R. M. yeast in the usual manner, 10 plates were dotted at 4 places with the bacterium, 10 plates were streaked at 2 places with the bacterium, and the remaining 10 plates were not inoculated with R. M. yeast or the bacterium. After 7 weeks the control plates had an average of 7.5 colonies/million spores inoculated, the plates containing the 4 colonies of the bacterium had 4.6 colonies/million spores inoculated, the streaked plates had 7.5 colonies/million spores inoculated, and the plates having no yeast or bacterium had no colonies. This demonstrated that this bacterium can quantitatively replace the function of the R. M. yeast.

2. *Thiamine:* Concentrations of 75, 100 and 125 μg/liter were attained in the following media: malt extract agar, Magenmaltz agar, medium A lacking yeast extract, and medium A lacking yeast extract and peptone. In one instance 10 concentrations of thiamine (from 50 up to 500 μg/liter) were attained in malt extract agar. In these experiments no colonies of *C. gigantea* appeared except for one colony in one of 10 plates containing malt extract, 350 μg/liter thiamine, and spores of *C. gigantea* #1400 C. This plate did not contain contaminating bacteria.

3. *Pyridoxine:* Concentrations of 50, 100 and 200 μg/liter were attained in medium A lacking peptone and yeast extract. No colonies of *C. gigantea* were observed.

4. *Inositol:* Concentrations of 50, 100 and 200 μg/liter were attained in medium A lacking yeast extract and peptone. No colonies of *C. gigantea* were observed.

5. *Riboflavin:* Concentrations of 10, 20 and 40 μg/liter were attained in malt extract agar. No colonies of *C. gigantea* were observed.

In each of the five above instances where vitamins were used in an attempt to replace the R. M. yeast, germination occurred in malt extract agar plates inoculated with R. M. yeast (controls). Thus, the effect of the yeast was not replaceable with these vitamins.

DISCUSSION

It is not difficult to postulate why our early attempts in these investigations to germinate *C. gigantea* spores met with failure. In most cases the experimental spores had been stored at room temperature, or higher,

quite often for long periods of time. More than likely the vast majority of these sporophores contained spores that were not viable. Furthermore it was expected that if certain treatments were successful then spore germination could be detected in a matter of days or at the most, less than 30 days. Such an assumption was incorrect. Also, it was not expected at the time that should germination occur, it would be less than 100 spores per million.

Once spores had been germinated in barley extract broth, it became apparent that some of these early notions were faulty. The barley method left a great deal to be desired so far as understanding the conditions necessary for germination. However, it suggested that spore germination of *C. gigantea* (a) requires periods in excess of 30 days to produce visible colonies, (b) occurs at a very low percentage, and (c) was possible only with spores from certain sporophores. The diversity of broths in which the spores germinate was and still is a puzzling matter. Their only apparent similarity is that all are decoctions of natural products.

When spores from *C. gigantea* were germinated in malt extract agar, a living yeast was necessary as a co-culture. The effect of the yeast raises many intriguing questions. Since various vitamins did not replace the yeast, it appears doubtful that the yeast has the sole effect of supplying a vitamin required for germination. However, a vitamin could be necessary as a stimulative agent with the yeast performing a second function—such as neutralizing or masking an inhibitor.

Chloromycetin was of great value in these experiments as a bacterial inhibitor. Apparently various bacteria occur naturally in the gleba of *C. gigantea* sporophores. Although some fungal contamination is present, by using in excess of ten replicates for each experiment, a sufficient number of plates free from these contaminants can be obtained.

Future papers in this series will deal with conditions necessary for germination of *C. gigantea* spores, tumor-retarding activity of cultures derived from spore germination, and the germination of basidiospores from genera closely related to *Calvatia*.

SUMMARY

The basidiospores of *Calvatia gigantea* have been germinated in (a) 6 different broth media, and, (b) malt extract agar inoculated with *Rhodotorula mucilaginosa* var. *sanguinea*.

The per cent germination is less than 0.001, the yeast can be replaced by a bacterium, spores from only certain sporophores germinate, incubation of at least 4 weeks is required to observe visible colonies, and in

malt extract agar (the most suitable for germination) seeding the spores *into* the medium appears to increase the germination rates.

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A PRELIMINARY REPORT OF THE MYXOMYCETES OF SOUTHERN ARIZONA

ADELAIDE E. EVENSON

The Myxomycetes, or Mycetozoa, are characteristically associated with the forests and grasslands of the more humid areas of the world. There appear to be no publications concerned with studies of the types of slime molds that are able to live under the peculiar climatic conditions existing in southern Arizona, nor have such studies been made in the arid regions of other parts of the world. It seemed appropriate, therefore, to make a preliminary report of the species of slime molds that have been found to occur in the arid southwest.

The studies reported herein are concerned with the myxomycetes that have been observed in an area that is within a radius of 90 miles of Tucson, Arizona. This area includes the landscaped parts of the city as well as the forest, chaparral, desert grassland and desert shrub zones which differ from one another in elevation, amount of precipitation and vegetation. Brief comparative data regarding these zones are given below:

Zone	Elevation	Annual precipitation	Vegetation
Forest	7–9000 ft	20–25 inches	Ponderosa pine, Douglas fir, etc.
Chaparral	4–5500 ft	12–15 inches	Oak, juniper, manzanita, etc.
Desert grassland	3–4500 ft	14–18 inches	Grasses, mesquite, yucca, oak, etc.
Desert shrub[1]	2–3000 ft	9–11.5 inches	Cacti, bur sage, palo verde, creosote, cat-claw, etc.

[1] The city of Tucson lies in this zone but its vegetation pattern has been altered by man's purposeful planting. Maintenance of this pattern requires heavy irrigation to supplement the annual rainfall.

The majority of the species found in the higher elevations of the Santa Catalina Mountains were represented by collected specimens that had fruited under natural conditions but only one specimen from the desert shrub zone and only two from the landscaped areas of the city were obtained in this manner. In my experience, the largest number of apparently freshly formed sporangia have been found in the forests

of these mountains from September through November. However, the sporangia of *Lamproderma Sauteri* and *Lepidoderma Carestianum* were observed fruiting in April in large numbers on twigs and dead leaves on the floor of a canyon near a stream. Midsummer collections made during the rainy season (July and August) have yielded few specimens other than *Fuligo septica, Lycogala epidendrum* and *Ceratiomyxa fruticulosa,* all of which occur in abundance at this time. The single collected species from the desert shrub zone, *Badhamia macrocarpa,* was found in March on decaying yucca near Oracle Junction, shortly after a freak snow storm. The two species from the cultivated area of the city were *Badhamia panicea* and *Fuligo septica* which fruited respectively on the stems of an oleander bush and on a lawn.

A few of the species from the forest areas and the majority of those from the other vegetation zones were cultivated in moist chambers from living and decaying plant material. In some instances, plasmodia or even fruiting structures appeared within 24 hours. In other cases, success might be delayed for days, or weeks, or even months. At times, plasmodia or sporangia appeared only after substrate had been repeatedly subjected over a period of 10 to 12 months to alternate and often prolonged periods of moistening and drying. There were also instances in which the presence of slime molds was not observed during long periods of the usual moist chamber cultivation but was noted when the substrate was permitted to dry and was subsequently subjected to very brief steaming (3–5 minutes) in the Arnold sterilizer to eliminate mites or other forms of animal life.

The 63 species of myxomycetes that have been collected or have been grown in moist chambers are listed with notes regarding the substrate on which fruiting occurred. The designation in parenthesis (mc) indicates that the specimen developed in moist chambers. The middle letter of the identifying number refers to the vegetation area from which the organism came, i.e., M (ponderosa pine or forest), D (desert shrub), G (desert grassland), C (chaparral), and L (landscaped).

CERATIOMYXACEAE:

Ceratiomyxa fruticulosa (Müll.) Macbr. A-M-8. On log of *Pinus ponderosa.*

LICEACAE:

Licea Kleistobolus Martin. A-M-64 (mc). Bark from living *Pinus ponderosa.*

Licea fimicola Dearness & Bisby. A-D-2 (mc). Decaying desert debris.

RETICULARIACEAE:

Lycogala epidendrum (L.) Fries. A-M-15. On log of *Pinus ponderosa.*

CRIBRARIACEAE:

Dictydium cancellatum (Batsch) Macbr. A-M-13. On log of *Pinus ponderosa.*

DIANEMACEAE:

Calomyxa[2] *metallica* (Berk.) Nieuwl. A-M-27. On log of *Pinus ponderosa.*

TRICHIACEAE:

Perichaena depressa Libert. A-D-5 (mc). On desert debris. Also observed in moist chamber cultures of bark from living *Tamarix gallica,* bark from living *Prosopis velutina* and decaying cholla (*Opuntia* sp.) from the desert shrub zone, on decaying leaves of *Nerium* sp. from landscaped areas, on bark of living *Quercus emoryi* from the chaparral zone, on bark of living *Juniperus deppeana* and *Prosopis velutina* from the desert grassland zone.

Perichaena vermicularis (Schw.) Rost. A-D-4 (mc). On decaying cactus debris (*Opuntia* sp. and *Carnegiea gigantea*). Also on decaying pine bark from ponderosa pine zone.

Perichaena corticalis (Batsch.) Rost. A-D-3 (mc). From various forms of decaying desert plants such as *Opuntia engelmanii* and *Carnegiea gigantea.* From the bark of living *Quercus gambelli* collected in the ponderosa pine zone.

Perichaena chrysosperma (Currey) Lister. A-D-8. On desert debris.

Arcyria incarnata (Pers.) Pers. A-M-12. On decaying log of *Pinus ponderosa.*

Arcyria insignis Kalchbr. & Cooke. A-M-62. On stump of *Pinus ponderosa.* A-D-11 (mc). Desert debris.

Arcyria nutans (Bull.) Grev. A-M-7 (mc). On decaying wood.

Arcyria cinerea (Bull.) Pers. A-D-19 (mc). From desert debris. Also noted in moist chamber cultures of bark from *Quercus emoryi* from the chaparral zone and needles of *Abies concolor* from the forest area.

Trichia botrytis (J. F. Gmel.) Pers. A-M-33. On decaying log of *Pinus ponderosa.*

Trichia persimilis P. Karst. A-M-18 (mc). On decaying wood. Also on *Cladonia rangiferina* (mc).

[2] G. W. Martin. Brittonia **13**: 109–113, 1961.

Trichia pusilla (Hedw.) Martin. A-M-1. On decaying log of *Pinus ponderosa.*

Trichia varia (Pers.) Pers. A-M-19. On decaying log of *Pinus ponderosa.*

Trichia affinis De Bary. A-M-76. On decaying log of *Pinus ponderosa.* A-C-21 (mc). Decaying vegetation.

Hemitrichia serpula (Scop.) Rost. A-M-30. On decaying log of *Pinus ponderosa.*

Hemitrichia stipitata (Massee) Macbr. A-M-9. On decaying log of *Pinus ponderosa.*

Hemitrichia vesparium (Batsch) Macbr. A-M-60. On decaying log of *Pinus ponderosa.*

Hemitrichia clavata (Pers.) Rost. A-M-25. On decaying log of *Pinus ponderosa.*

ECHINOSTELIACEAE:

Echinostelium minutum De Bary. A-M-65 (mc). Bark of living *Pinus ponderosa.*

STEMONITACEAE:

Stemonitis axifera (Bull.) Macbr. A-M-50. On decaying log of *Pinus ponderosa.*

Stemonitis fusca Roth. A-M-74. On decomposing wood.

Stemonitis pallida Wingate. A-M-14. On decomposing wood.

Stemonitis virginiensis Rex. A-M-11. On decaying log of *Pinus ponderosa.*

Stemonitis flavogenita Jahn. A-G-2 (mc). On bark of living *Prosopis velutina.*

Comatricha aequalis Peck. A-M-17. On decaying log of *Pinus ponderosa.*

Comatricha irregularis Rex. A-M-70. On decaying log of *Pinus ponderosa.*

Comatricha lurida Lister. A-M-35 (mc). On bark of living *Pseudotsuga taxifolia.* Also observed in moist chamber cultures of the bark of *Quercus emoryi* from the desert grassland zone.

Comatricha nigra (Pers.) Schroet. A-M-69. On decaying log of *Pinus ponderosa.*

Comatricha tenerrima (M. A. Curt.) G. Lister. A-M-34. On stump of *Pinus ponderosa.*

Comatricha typhoides (Bull.) Rost. A-M-64 (mc). On decaying wood.

Comatricha laxa Rost. A-M-5. On decaying *Pinus ponderosa* log. A-G-1. On bark of living *Populus fremontii.*

Comatricha pulchella (C. Bab.) Rost. A-D-1 (mc). On desert debris.

Clastoderma Debaryanum A. Blytt. A-M-46. On decomposing wood.

Lamproderma arcyrionema Rost. A-M-10. On decaying *Pinus ponderosa* log.

Lamproderma Sauteri Rost. A-M-28. On dead twigs and leaves on the ground.

PHYSARACEAE:

Fuligo septica (L.) Weber. A-M-6. On decaying log. A-L-27. On lawn in city.

Badhamia macrocarpa (Ces.) Rost. A-D-18 and A-D-14 (mc). Common on many types of desert vegetation both living and dead. Also observed in moist chamber cultures of bark from *Quercus gambelli* from ponderosa pine zone.

Badhamia decipiens (M. A. Curt.) Berk. A-M-80 (mc). On bark of living *Quercus gambelli.*

Badhamia panicea (Fries) Rost. A-D-6 (mc). On dead cholla (*Opuntia* sp.). A-L-12. On living oleander (*Nerium* sp.).

Physarum galbeum Wingate. A-M-79. On decaying log of *Pinus ponderosa.*

Physarum notabile Macbr. A-M-4. On decaying wood. Also on decaying cholla from the desert shrub zone.

Physarum nutans Pers. A-M-55. On decaying wood.

Physarum cinereum (Batsch) Pers. A-M-26 (mc). Needles of living *Pinus ponderosa.* Also observed in moist chamber culture of debris from the chaparral zone.

Physarum pusillum (Berk. & Curt.) G. Lister. A-D-10 (mc). On decaying leaves of *Populus fremonti.* Also frequently observed on other types of desert debris and in various types of decaying vegetation from the desert grasslands.

Physarum compressum Alb. & Schw. A-D-16 (mc). On desert debris.

Physarum mutabile (Rost.) G. Lister. A-M-68. On *Pinus ponderosa* bark. A-D-17 (mc). On decaying saguaro ribs.

Physarum tenerum Rex. A-M-77. On decaying *Pinus ponderosa* log.

Craterium minutum (Leers) Fries. A-M-31. On decomposing wood.

Leocarpus fragilis (Dicks.) Rost. A-M-2. On stump of *Pinus ponderosa.*

DIDYMIACEAE:

Diderma radiatum (L.) Morgan. A-M-61. Within a decaying log of *Pinus ponderosa.*

Diderma trevelyani (Grev.) Fries. A-M-75. On decaying wood.

Mucilago spongiosa (Leysser) Morgan. A-M-20. On decaying wood.

Didymium vaccinum (Dur. & Mont.) Buchet. A-G-3 (mc). On bark of living *Juniperus deppeana.*

TABLE I

MYXOMYCETE SPECIES DISTRIBUTION IN THE VARIOUS VEGETATION AREAS

Species	Vegetation area				
	Forest	Chaparral	Desert grassland	Desert shrub	Landscaped areas in city
Ceratiomyxa fructiculosa	×	—	—	—	—
Licea Kleistobolus	×	—	—	—	—
Licea fimicola	—	—	—	×	—
Lycogala epidendrum	×	—	—	—	—
Dictydium cancellatum	×	—	—	—	—
Calomyxa metallica	×	—	—	—	—
Perichaena depressa	—	×	×	×	×
Perichaena vermicularis	×	—	—	×	—
Perichaena corticalis	×	—	—	×	—
Perichaena chrysosperma	—	—	—	×	—
Arcyria incarnata	×	—	—	—	—
Arcyria insignis	×	—	—	×	—
Arcyria nutans	×	—	—	—	—
Arcyria cinerea	×	×	—	×	—
Trichia botrytis	×	—	—	—	—
Trichia persimilis	×	—	—	—	—
Trichia pusilla	×	—	—	—	—
Trichia varia	×	—	—	—	—
Trichia affinis	×	×	—	—	—
Hemitrichia serpula	×	—	—	—	—
Hemitrichia stipitata	×	—	—	—	—
Hemitrichia vesparium	×	—	—	—	—
Hemitrichia clavata	×	—	—	—	—
Echinostelium minutum	×	—	—	—	—
Stemonitis axifera	×	—	—	—	—
Stemonitis fusca	×	—	—	—	—
Stemonitis pallida	×	—	—	—	—
Stemonitis virginiensis	×	—	—	—	—
Stemonitis flavogenita	—	—	×	—	—
Comatricha aequalis	×	—	—	—	—
Comatricha irregularis	×	—	—	—	—

Didymium dubium Rost. A-D-15 (mc). Decaying cholla debris. Also observed on debris from the desert grasslands and chaparral areas.

Didymium anellus Morgan. A-D-20 (mc). On dead *Acacia constricta*. Common on decaying desert material. Also observed in moist chamber preparations of dead plant material from the chaparral zone.

Didymium minus (Lister) Morgan. A-M-67 (mc). On decaying *Pinus ponderosa*.

TABLE I—*Continued*

Species	Vegetation area				
	Forest	Chaparral	Desert grassland	Desert shrub	Landscaped areas in city
Comatricha lurida	×	—	×	—	—
Comatricha nigra	×	—	—	—	—
Comatricha tenerrima	×	—	—	—	—
Comatricha typhoides	×	—	—	—	—
Comatricha laxa	×	—	×	—	—
Comatricha pulchella	—	—	—	×	—
Clastoderma Debaryanum	×	—	—	—	—
Lamproderma arcyrionema	×	—	—	—	—
Lamproderma Sauteri	×	—	—	—	—
Fuligo septica	×	—	—	—	×
Badhamia macrocarpa	×	—	—	×	—
Badhamia decipiens	×	—	—	—	—
Badhamia panicea	—	—	—	×	×
Physarum galbeum	×	—	—	—	—
Physarum notabile	×	—	—	×	—
Physarum nutans	×	—	—	—	—
Physarum cinereum	×	×	—	—	—
Physarum pusillum	—	—	×	×	—
Physarum compressum	—	—	—	×	—
Physarum mutabile	×	—	—	×	—
Physarum tenerum	×	—	—	—	—
Craterium minutum	×	—	—	—	—
Leocarpus fragilis	×	—	—	—	—
Diderma radiatum	×	—	—	—	—
Diderma trevelyani	×	—	—	—	—
Mucilago spongiosa	×	—	—	—	—
Didymium vaccinum	—	—	×	—	—
Didymium dubium	—	×	×	×	—
Didymium anellus	—	×	—	×	—
Didymium minus	×	—	—	—	—
Didymium melanospermum	×	—	—	—	—
Lepidoderma Carestianum	×	—	—	—	—
Totals	52	6	7	16	3

Didymium melanospermum (Pers.) Macbr. A-M-78. On decaying wood.

Lepidoderma Carestianum (Rab.) Rost. A-M-29. On twigs and leaves on the ground.

TABLE I lists the species that have been observed in each of the different vegetation zones and in the landscaped areas of the city.

The greatest variety of species has been found in the forested areas where the annual rainfall is heaviest. The lack of correlation between the numbers of species and the amount of rainfall in the zones at lower elevations may reflect simply the amount of study that has been devoted to each. Much more attention has been given to the desert shrub and the forested regions than to the others. It should be pointed out that, although relatively few species have so far been observed, myxomycetes are exceedingly common in moist chamber cultures of desert plant material. In my experience, the most frequently occurring species have been *Badhamia macrocarpa, Perichaena depressa, P. vermicularis* and *P. corticalis.*

Investigation into this phase of arid lands biology is continuing.

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STEREUM TAXODII IN JAPAN AND FORMOSA

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(WITH 13 FIGURES)

INTRODUCTION

For 60 years, taxonomists who worked with stereoid fungi identified specimens of *Stereum sulcatum* Burt (1) with great confidence, for no other species seemed to be so unquestionably unique. Then Davidson, Toole, and Campbell (3) ended the idyl by publishing their preliminary note on the cause of pecky cypress decay. Those authors reported that a *Stereum* basidiocarp was associated with the decay and that cultures from the basidiocarp were identical with cultures isolated from decayed wood of *Taxodium distichum* (L.) Rich. Subsequent study (2) of the basidiocarp revealed its very close resemblance to those of *S. sulcatum*, but it grew in the top part of the tree instead of the basal area; it was associated with a rot much different from that caused by *S. sulcatum;* and the hymenial surface was dull instead of bright. Most perplexing was the discovery that it differed greatly from *S. sulcatum* in cultural characteristics. Recognition of that difference, together with differences in the morphology of the basidiocarp, led to the realization that there are at least two distinct stereoid species entirely different from the others but astonishingly alike.

In a publication by Davidson, Lentz, and McKay (2) the *Stereum* from *Taxodium* was described as *S. taxodii* Lentz & McKay. Before the description was published, Toole had collected several additional specimens of the fungus, all associated with pecky decay of bald cypress and all identical with the original collection in pertinent characteristics of the basidiocarps and cultures. Specimens of *S. taxodii* listed in the original description were from Florida, Louisiana, Mississippi, and Texas, and cultures were obtained from Florida, Georgia, Mississippi, and

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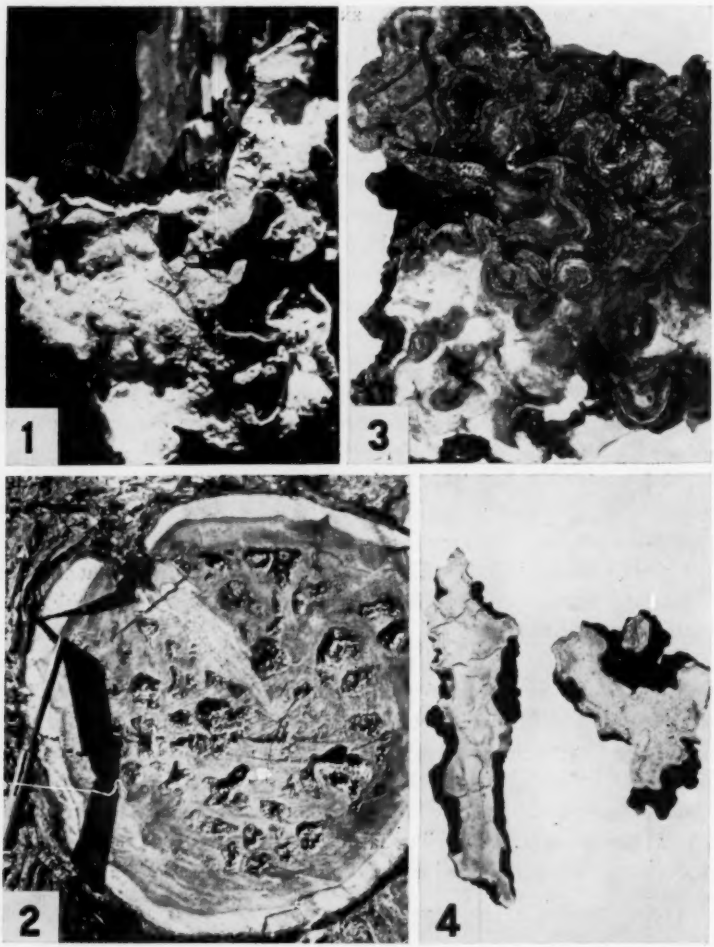
North Carolina. Only a few days before publication of the type description, an additional specimen was discovered by Aoshima and Lentz in the L. O. Overholts herbarium (no. *14976*) at Pennsylvania State University. Overholts had recognized the affinity of that collection to *S. sulcatum* but noted that the context appearance was divergent. The



FIGS. 1–4. *Stereum taxodii.* FIG. 1. Basidiocarp on *Cryptomeria japonica* (printed from color transparency), × 1½. FIG. 2. Lotus-stem decay in log of *C. japonica* (printed from color transparency), × ⅞. FIG. 3. Basidiocarp from *Chamaecyparis formosensis,* × 1. FIG. 4. Basidiocarp from *Torreya nucifera,* × ¾.

specimen in the Overholts herbarium had been collected in South Carolina by G. G. Hedgcock in 1921. Overholts called it *Stereum calceum* but did not publish the name. His specimen has a peculiar milky-white hymenial coloration that apparently influenced his choice of a specific epithet.

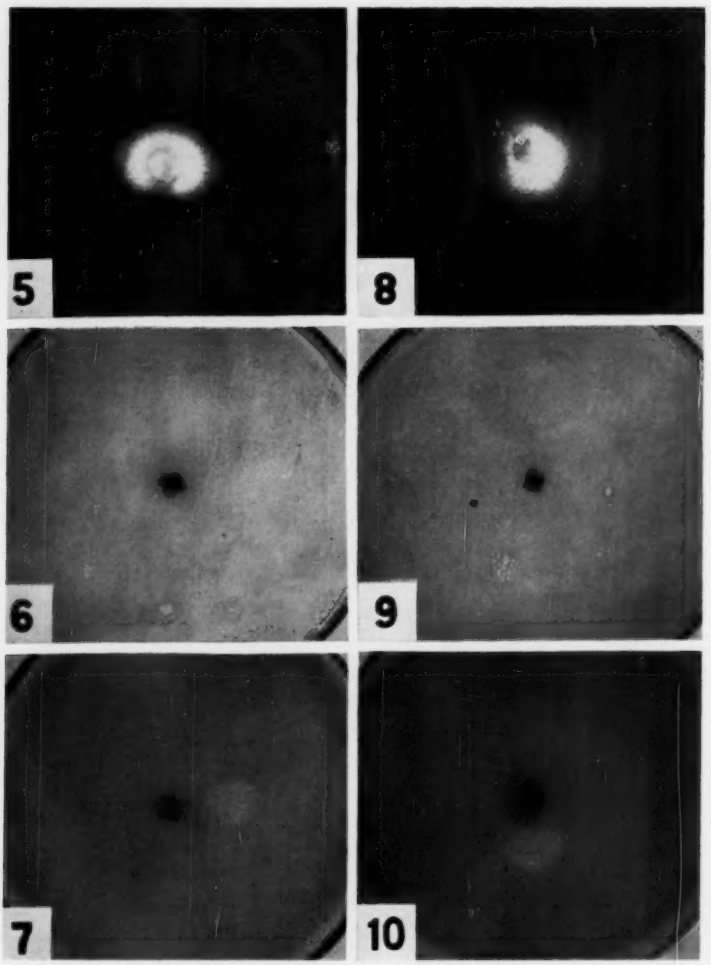
In the United States, no specimen of *S. taxodii* has been collected from a host other than *Taxodium*. However, a short time before publication of the type description of *S. taxodii*, correspondence between Davidson and Aoshima resulted in the discovery that the same species might be found outside the southern United States and on a host other than *Taxodium*.

STEREUM TAXODII FROM CRYPTOMERIA

In September 1959, a severe typhoon felled or damaged many trees in Japan. Among the trees thus affected were many old specimens of *Cryptomeria japonica* (L.f.) D. Don, a conifer closely related to *Taxodium*. At Mie-ken in central Japan, a forest of venerable *Cryptomeria* trees surrounds the famous Shinto shrine known as the Ise Shrine. During the typhoon of 1959, many of these old trees were uprooted, and large branches were broken off others. While salvaging the fallen trees, loggers discovered decay of a kind which was already well known among Japanese foresters as "lotus-stem" decay of *Cryptomeria* (FIG. 2). In Japan, lotus-stem decay was thought to be caused by *Fomes ulmarius* (Fr.) Gill., just as the pecky decay of *Taxodium* in the United States was formerly attributed to *F. ulmarius* (**5**). But lotus-stem decay and pecky cypress decay both appear in the upper areas of the host trees, whereas *F. ulmarius* has been found only in connection with butt rots of many woody hosts, including *Taxodium* and *Cryptomeria*.

Examination of the infected *Cryptomeria* trees revealed basidiocarps of a *Stereum* (FIG. 1) developing on branch stubs in the upper crown or bole areas. The decay-producing organism seemed to have entered the trees through the branch stubs. A culture isolated from lotus-stem decay was sent to Beltsville, where it was compared with three cultures obtained from pecky cypress decay and three from basidiocarps of *Stereum taxodii*. The culture from *Cryptomeria* was essentially identical with the six from *Taxodium*, but lack of monosporous isolates of the fungus from Japan prevented verification of the relationship by interfertility tests. A basidiocarp, collected from one of the *Cryptomeria* trees and compared with specimens obtained from bald cypress, was determined as *S. taxodii*. A similar *Stereum* and specimens of decay were collected from large *Cryptomeria* trees near Tokyo in the summer

of 1960. In all instances, the decay was confined to the upper parts of the tree trunks. Discovery of *S. taxodii* in Japan resulted in a search for additional records, and it was found that the same species had been collected previously by K. Hara in a natural monument preserve of *Cryptomeria* in Kochi-ken. That collection, reported by Sasaki (7) as



FIGS. 5–10. *Stereum taxodii* cultures at 14 days. FIGS. 5, 6, 7. Isolate from *Cryptomeria japonica* on diamalt, gallic acid and tannic acid media, respectively. FIGS. 8, 9, 10. Isolate from *Taxodium distichum* on diamalt, gallic acid and tannic acid media, respectively. All × ⅔.

S. sulcatum, was one of the two specimens examined as a basis for preparation of the following notes on *S. taxodii* from *Cryptomeria.*

BASIDIOCARPS AND CULTURES FROM CRYPTOMERIA

Basidiocarp Characteristics: Macroscopically basidiocarps (FIG. 1) of the fungus from *Cryptomeria* closely resemble specimens from *Taxodium* (**2**), but the hymenial coloration is intermediate between "pinkish buff"[4] and "avellaneous" and without the pronouncedly grayish cast noted in the type. Microscopic characteristics also are similar to those of specimens from *Taxodium.* Sizes of the various anatomical structures (FIG. 11, a, b, e, f) are within the ranges, but do not attain the maximums, given in the original description. The basidiospores (FIG. 11, g) are subglobose, minutely echinulate, hyaline, amyloid, 6–7.5 × (4.5–)5–6 μ.

Specimens Examined: Japan—Kochi-ken (coll. K. Hara, February 1955), Mie-ken (coll. K. Aoshima, December 1959).

Cultural Characteristics: In culture (FIGS. 5–7), the fungus causing lotus-stem decay of *Cryptomeria* in Japan is practically identical macroscopically and microscopically with cultures of *S. taxodii* from *Taxodium distichum* in the southeastern United States (FIGS. 8–10). There is no essential difference in growth rate; the same olive-yellow stain surrounding the mat is a conspicuous feature; the staining hyphae (FIG. 11, c) have numerous large clamp-connections; the fiber hyphae (FIG. 11, d) are abundant and characteristic; reactions on gallic acid (FIG. 6) and tannic acid (FIG. 7) media are typical; and chlamydospores (FIG. 11, h) are similar in size and shape to those in culture from *Taxodium* but develop late and only sporadically.

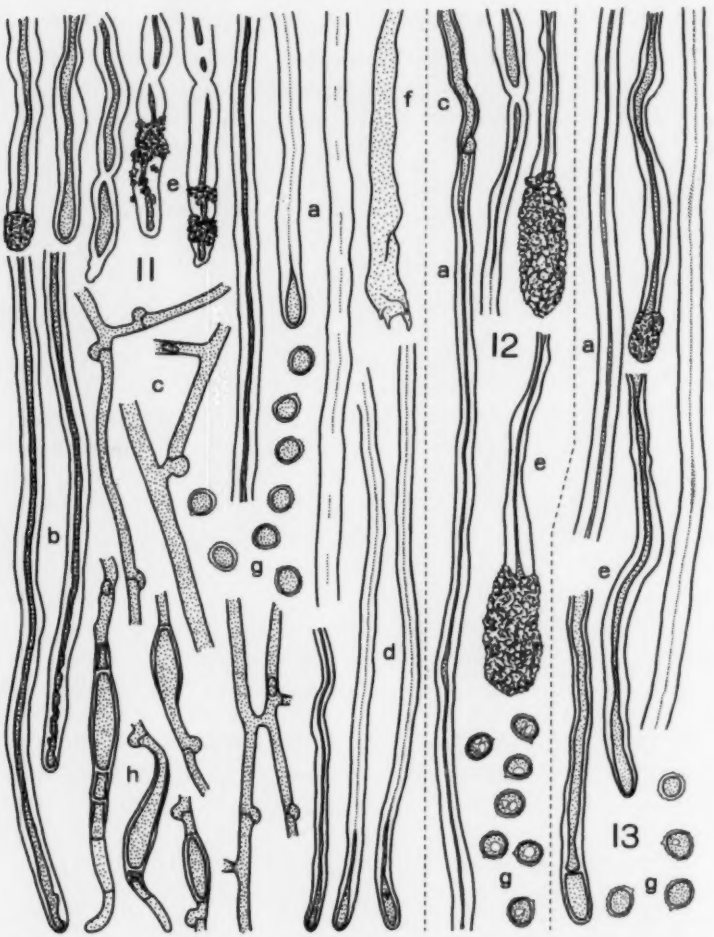
Culture Studied: Japan—Mie-ken, Government Forest Expt. Sta., Forest Mycology Lab., Meguro, Tokyo, Japan (S-36-b, isolated in December 1959, by K. Aoshima, from 200-year-old tree).

STEREUM TAXODII FROM TORREYA AND CHAMAECYPARIS

Many years ago, Yamamoto and Ito (**9**) described a rot of *Chamaecyparis formosensis* Matsum., which they attributed to *S. sulcatum.* The fungus was said to attack the trunk and branches of living trees in Formosa and to cause a heartwood rot. Illustrations (Yamamoto and Ito, FIG. 1, A) reveal that the rot is the now-familiar kind found in both *Taxodium* and *Cryptomeria.* Yamamoto and Ito described the decay as a "white pocket rot," but their terminology was based on an attempt

[4] Color terms enclosed by quotation marks are derived from Ridgway (**6**).

to distinguish the decay from a typical brown rot and not to define it as a typical white rot. The term "renkon-kusare," which they used, is



FIGS. 11–13. *Stereum taxodii* anatomical elements drawn with the aid of a camera lucida attachment. FIG. 11. Elements of basidiocarp (a, b, e, f, g) and culture (c, d, h) from *Cryptomeria japonica;* a. Skeletal hyphae, b. Tomental hyphae, c. Generative hyphae, d, Fiber hyphae, e. Cystidia, f. Collapsing basidium, g. Spores, h. Chlamydospores. FIG. 12. Elements of basidiocarp from *Torreya nucifera;* a. Skeletal hyphae, c. Generative hypha, e. Cystidia, g. Spores. FIG. 13. Elements of basidiocarp from *Chamaecyparis formosensis;* a. Skeletal hyphae, e. Cystidia, g. Spores. All × 650.

translatable as "lotus-stem rot" and apparently denotes the kind of decay found in *Taxodium* and *Cryptomeria*. Photographs given to Aoshima by Ito show basidiocarps of a *Stereum* growing from branch stubs high in the crown of a *Chamaecyparis* tree. Cultures of the fungus from *Chamaecyparis* are not yet available for study, but in morphology and growth habit that fungus rather closely resembles *S. taxodii*.

At least three Japanese authors in addition to Yamamoto and Ito have published reports purportedly of *S. sulcatum*. Imazeki (**4**) included that species among his hard and perennial Stereums from Japan and listed *Chamaecyparis* and *Torreya* as the hosts. His report of *Torreya* as a host seems to have been based on a collection by Yasuda (**10**), which was also mentioned in the paper by Yamamoto and Ito. Teramoto (**8**) reported the occurrence of *S. sulcatum* on *Torreya* and a coniferous stump in Japan and on *Chamaecyparis formosensis* from Formosa. The specimen reputed to have been collected from the coniferous stump was lost after the publication of Teramoto's paper, and there is no certainty that it was actually collected from a stump. A specimen from *Torreya nucifera* Sieb. & Zucc., collected by Y. Hayashi and now in the National Fungus Collections, obviously is not *S. sulcatum*. Although final verification of the identities of specimens from *Torreya* and *Chamaecyparis* is deferred until cultures are available, basidiocarp morphology closely resembles that of *S. taxodii* and causes us to believe that the specimens belong in that species.

BASIDIOCARPS FROM TORREYA

The specimen from *Torreya* (FIG. 4) consists of two moderately thick basidiocarps, each somewhat larger than any yet collected from *Taxodium*. The upper surface is hard, blackish, and glabrous in the older area near the umbos but has a brownish tomentum toward the margin. The hymenium is smooth and "pale pinkish buff." Microscopically, the collection from *Torreya* is much like other specimens of *S. taxodii;* hence there seems to be no reason for hesitation in referring it to that species. The appearance in section is quite characteristic, with serried hyphae (FIG. 12, a, c) giving an appearance entirely distinct from that observed in *S. sulcatum*. There are numerous thick-walled cystidia (FIG. 12, e) 55–86 × 6–8 μ, with the apices enlarged to 10.5–13 μ and either nonencrusted or scantily to heavily encrusted. The spores (FIG. 12, g) are practically identical with those of basidiocarps from *Taxodium* but do not attain the maximum size of the latter. The range is 6–7 × 5–6.5 μ.

Specimen Examined: Japan—Shizuoka-ken (Japanese Forest Path. *2605*, coll. July 22, 1950, by Y. Hayashi).

BASIDIOCARPS FROM CHAMAECYPARIS

The specimens from *Chamaecyparis* are more difficult to interpret than any of the other collections. One large piece of basidiocarp (FIG. 3) is 8 × 6 cm in extent and 4 cm thick from point of attachment to the margins of the proliferously multiple pilei. The upper surface is blackish, hard, glabrous, and sulcate, and the margins are thick and sulcate. The hymenial surface is "pale pinkish buff" in one specimen but somewhat darker in the other. A single pileus may be 3 mm thick, even where not excessively thickened by distorted and superimposed growth. Thick-walled encrusted and nonencrusted cystidia (FIG. 13, e) are present. The spores (FIG. 13, g) are very much like those of *S. taxodii*) from *Taxodium*, with a range of 6–7(–8) × (5–)6–6.5 μ. Interpretation of the nature of the hyphae in basidiocarps from *Chamaecyparis* is especially difficult. The arrangement is not very orderly and thus somewhat resembles that of *S. sulcatum*. But careful examination reveals that the twisted and interwoven hyphae are thin- to thick-walled generative hyphae with clamp-connections. The relatively slender, very thick-walled, short-branched hyphae so prevalent in *S. sulcatum* are probably lacking. Skeletal hyphae (FIG. 13, a) are somewhat more twisted than in specimens of *S. taxodii* from other hosts, but their contortions are caused by the distorting pressure of the robust branched and twisted generative hyphae of the context.

Specimens Examined: Formosa (two collections, comm. T. Ito, September 9, 1960).

CONCLUSIONS

All reports of *S. sulcatum* from Japan are probably based on specimens of *S. taxodii*. Differences in basidiocarp form and hymenial coloration and slight differences in hyphal, cystidial, and spore size and appearance on the various hosts do not seem of great significance. Instead, these characteristics are revealed as considerably more variable than could be visualized by study of specimens from *Taxodium* alone.

Although *S. taxodii* is somewhat less morphologically homogeneous after inclusion of the Asian specimens, and will certainly require additional taxonomic study, several well-marked characteristics clearly separate it from *S. sulcatum*. Among these, the most distinctive are (1) the nature of the decay and the position of the basidiocarp on the host; (2)

the kinds and arrangement of hyphae that form the basidiocarp, and the sizes of basidia and spores; (3) cultural characteristics, including presence or absence of chlamydospores and oedocephaloid conidiophores, hyphal characteristics, and presence or absence of extra-cellular oxidase enzymes as revealed by reaction on gallic acid and tannic acid media. In addition, the results of pairing single-spore isolates are apparently definitive (2). On the basis of these characters, the following distinctions may be drawn between *S. taxodii* and *S. sulcatum*.

		S. taxodii	*S. sulcatum*
Decay		White stringy to brown powdery rot in elongated pockets in tree top	White pocket to white stringy butt rot
Basidiocarp	Hyphae	(1) Branched generative hyphae with clamps; (2) skeletal hyphae	(1) Branched generative hyphae with clamps; (2) skeletal hyphae; (3) short- and profusely-branched, slender, thick-walled hyphae that bind the context hyphae together
	Basidia	50–75 × 8 μ	25–35 × 4.5–6 μ
	Spores	Amyloid, usually minutely echinulate, 6–8.5 × 5–7 μ	Amyloid, usually minutely echinulate, 5.5–6.5 × 4.5–5.5 μ
Culture	Conidiophores	None	Oedocephaloid
	Chlamydospores	Oblong-ellipsoidal, with walls slightly thickened	None
	Oxidase reaction	Weak or negative	Positive
	Fiber hyphae	2.5–6 μ in diameter	(1.5–)2(–3) μ in diameter

SUMMARY

Stereum taxodii, recently described from *Taxodium distichum* in the southern United States, occurs also on *Cryptomeria japonica* in Japan. Specimens from *Torreya nucifera* and *Chamaecyparis formosensis* in Japan and from *C. formosensis* in Formosa also seem to be *S. taxodii*. Occurrence of *S. taxodii* on *Cryptomeria* was established by examination of basidiocarps and cultures and on *Torreya* and *Chamaecyparis* by examination of basidiocarps. *S. taxodii* differs from *S. sulcatum* in the type of decay that it causes, in basidiocarp anatomy and morphology, and in cultural characteristics.

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A BLUESTAIN IN BALSAM FIR[1]

RENÉ POMERLEAU AND D. E. ETHERIDGE

(WITH 13 FIGURES)

INTRODUCTION

The occurrence in North America of a bluestain in the heartwood of balsam fir (*Abies balsamea* (L.) Mill) has been recognized for over a quarter of a century, but attempts to identify the causal fungus have been unsuccessful because of failure to obtain the fruiting stage in culture (**2**, **3**, **7**). Recently, it was possible to identify the fungus isolated from bluestained wood by comparison with cultures obtained from ascospores of *Amphisphaeria thujina* (Peck) Sacc. collected on dead branches of balsam fir (**6**). Further studies have been undertaken on the fungus and the associated stain. The present paper deals with the occurrence, taxonomy, and cultural characteristics of the fungus. In addition, some factors that may influence the establishment of bluestain and its relation with decay organisms have been investigated.

MATERIALS AND METHODS

Detailed information on the occurrence of bluestain was obtained from a critical examination of 200 living balsam fir trees in mature stands of the Dryopteris-Oxalis and Sphagnum-Oxalis forest types at Duchesnay, Quebec. Isolations to confirm the presence or absence of the fungus were made from branches at their point of juncture with the stem. Sections taken from the base of each branch were split through the centre with a sterile scalpel, and three small pieces of wood were taken from successive points, about ¼ inch apart, either along the branch axis or at right angles to it, and placed on 2.5% malt extract agar slants. The same technique for making isolations was followed for sampling broken tops and stem lesions, except that the isolations were more widely spaced. The age and year of death of branches were determined by counting the number of annual rings at the base of each branch, and comparing their age with those of the adjacent stem. The difference between these values was assumed to equal the number of years that had

[1] Contribution No. 803 from the Forest Entomology and Pathology Branch, Department of Forestry, Ottawa, Canada.

elapsed since the branches had died. The year of injury of broken tops and stem lesions was determined by counting the number of years since the formation of wound callus.

Six isolates of the bluestain fungus, representing three cultures obtained from ascospores and three from stained wood, were used in this investigation. Data about the cultures are given in TABLE I. Cultural studies on the fungus were carried out on 2.5% malt extract agar unless otherwise recorded. Specimens of the stained wood and of the fruiting stage were collected at Duchesnay, Quebec, in 1960.

Growth of the fungus at different temperatures was studied on plates, and the tests were made in duplicate at temperatures of 2.5, 11, 17, 22, 28, 32, and 36° C. Two diameter measurements of the colonies at right

TABLE I

SOURCE OF CULTURES

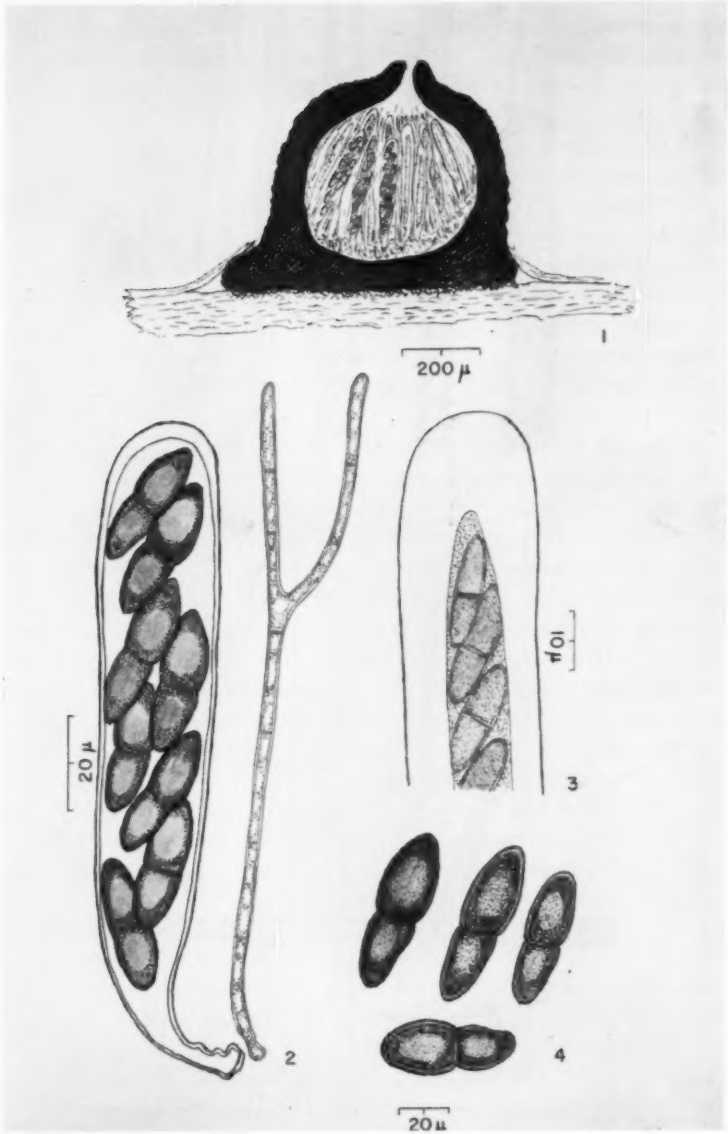
Culture No.		Host	Locality	Remarks
HPFQ	DAOM #			
292	75766	*Abies balsamea*	Duchesnay, Que.	Ascospores
293	75767	*Abies balsamea*	Duchesnay, Que.	Bluestained branch wood
294	75768	*Abies balsamea*	Duchesnay, Que.	Ascospores
295	75769	*Abies balsamea*	Duchesnay, Que.	Bluestained branch wood
296	75770	*Abies balsamea*	Duchesnay, Que.	Ascospores
126-C-2		*Abies balsamea*	Duchesnay, Que.	Bluestained branch wood

angles to each other were made at the end of 7 and 14 days. The diameter growth during the second week was used to calculate the mean daily growth of the cultures.

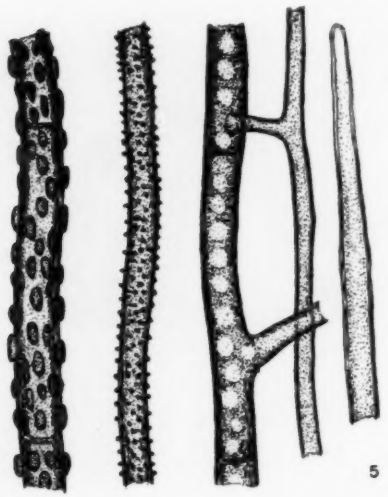
The minimal moisture requirements of the fungus were studied by a method recently developed in this Laboratory (**5**), which depends on the ability of the fungus to grow through wood of different moisture contents. The tests were made in triplicate, at room temperature (25° C), with small blocks of balsam fir heartwood kept under bell-jars in atmospheres of different relative humidities [2] to maintain equilibrium in wood moisture contents of 12, 15, 18, 21, 25, and 30% (o.d.w. basis).

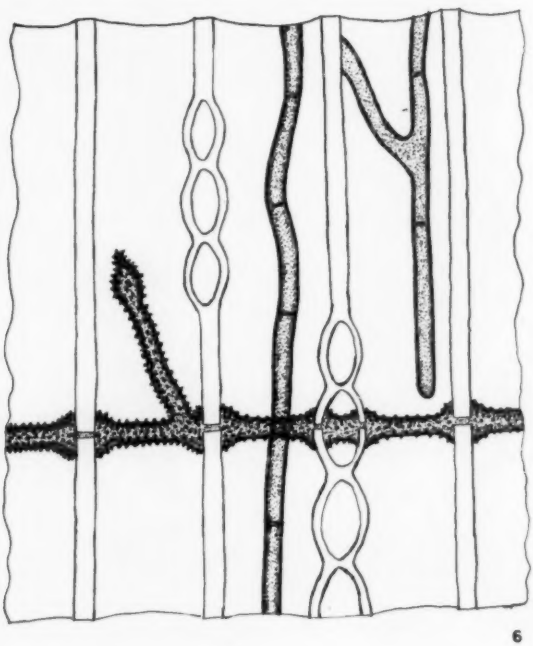
[2] Saturated solutions of the following salts were used for the control of humidity (the corresponding humidity and equilibrium moisture content values are given in brackets): $NaCl$–$NaNO_3$ (69%–12%), NaCl (75%–15%), KCl (85%–18%), BaCl (90%–25%), and water (100%–30%).

FIGS. 1–4. *Kirschsteiniella thujina*. 1. Cross section of the mature perithecium showing the hemisphaerical outline, the thick, homogeneous wall and the slightly immersed flat base. 2. Mature ascus and paraphysoid filament. 3. Immature ascus showing the thick wall and the lumen. 4. Mature ascospores.



FIGS. 1–4.





FIGS. 5, 6.

TAXONOMY AND DESCRIPTION OF THE FUNGUS

The fruiting stage of the fungus associated with bluestain has already been referred to *Amphisphaeria thujina* (**6**). However, the taxonomic position and a full description of this species have not appeared previously.

In 1875, Peck (**9**) proposed the name *Sphaeria thujina* to designate a fungus forming small carbonaceous fruiting bodies on decorticated, rotten wood of *Thuya occidentalis* L. collected in the Adirondack Mountains, New York. In 1882, this species was transferred to the genus *Amphisphaeria* by Saccardo (**11**). In their transcription of Peck's original description, Ellis and Everhart (**4**) stated that this fungus might be a *Diplodia* since no mention was made of asci. In order to compare the fungus found in Quebec with *A. thujina* and to clarify the taxonomy of the fungus described by Peck, the type collection was obtained and studied.

Although no mention was made of asci in the original description (a note by Peck, which accompanied the type collection also stated that no asci were seen), the material collected in the Adirondack Mountains included black perithecia with asci and bicellular, dark ascospores. A comparative study of the type collection and the Quebec material clearly demonstrated that both collections are identical. The perithecia are carbonaceous, hemispherical with a flat base, and only slightly immersed in the wood (FIGS. 1, 8, 9). The perithecial wall is rather thick and well-defined (FIG. 1). Asci of the two collections are thick-walled at first (FIG. 3), then bitunicate (FIGS. 2, 10), cylindrical or enlarged below, and of similar size. Ascospores of both the New York and Quebec collections are dark brown, bicellulate, the upper cell slightly attenuate at the end, and of the same size range (FIGS. 2, 4, 10). In both collections the asci are surrounded by numerous paraphysoid filaments (FIG. 2). Undoubtedly, the fungus causing the bluestain of balsam fir in Quebec is the same fungus that was named *A. thujina* by Peck, and by Saccardo.

Many species of *Amphisphaeria* have been described in Europe, in North America, and in other parts of the world. Most of them, however, were named after 1875, while those described earlier have either much smaller or much larger ascospores. Species such as *A. olearum* de Not. & Ces., *A. aethiops* (B. & C.) Sacc., *A. salebrosa* (C. & P.)

FIGS. 5, 6. *Kirschsteiniella thujina*. 5. Mycelium in culture showing young and old, smooth and incrusted hyphae. 6. Mycelium in wood vessels showing smooth and incrusted hyphae and constricted filaments across the cell walls.

Sacc, have spores of comparable size but were named after *A. thujina*. Unless a comparative study of all entities described in this genus is undertaken, it will be difficult to decide which species should be maintained or rejected. Therefore, the species of Peck is considered valid for the time being.

In 1863, Cesati and de Notaris (**1**) established the genus *Amphisphaeria* for species of the old genus *Sphaeria* which have two-celled, dark colored ascospores. According to Petrak (**10**), the diagnosis of this genus was incomplete and the type species is not known. Furthermore, Petrak has critically studied a few species and has shown that the genus comprises at least two groups of species, quite different in their morphological features. *A. umbrina* (Fr.) Ces. & de Not., which was designated the type species by Petrak, has perithecia deeply immersed in the bark with only the ostiolum protruding. The lower half of the perithecial wall is not sharply defined and is composed of filaments and particles of the substratum. On the other hand, he found that perithecia of *A. applanata* (Fr.) Ces. & de Not. are erumpent and their walls rather thick, dark, well-defined, and composed of filamentous and cellular elements without remnants of the substratum. Moreover, according to Petrak, the two species are different in that the asci are thin-walled in the former and thick-walled in the latter, whilst periphyses are present at the ostiolum in the former species and absent in the latter. Because of these major differences he proposed the new genus *Kirchsteiniella* to include *A. applanata* and other similar species. This view was recently upheld by Munk (**8**) and also by Schrantz (**12**) who added that, in *A. applanata*, the asci are bitunicate and without apical structure as in *A. umbrina* which has unitunicate asci with a complete apical structure.

Since the perithecia of *A. thujina* are superficial with thick, well-defined walls and the asci bitunicate as in *A. applanata* (FIGS. 1, 2, 10), this species should be transferred to the genus *Kirschsteiniella*. This necessitates a complete description of the species.

Kirschsteiniella thujina (Peck) Pomerleau & Etheridge, comb. nov. syn: *Sphaeria thujina* Peck, 27th Report of the Botanist, New York State Museum, p. 110, 1875; *Amphisphaeria thujina* (Peck) Sacc., Syll. Fung. 1: 226. 1882.

Perithecia gregarious or scattered, at first immersed under a thin layer of wood, soon superficial except the flat base slightly sunken in the wood, campanulate, with a papilliform or obtusely conical ostiolum which is closed at first and finally opened, 450–600 μ in diameter, 375–500 μ in height, slightly rugulose. Peridium 50–75 μ thick, carbonaceous

or brownish, composed of large isodiametrical cells and filaments. Asci cylindrical or enlarged at the lower half, rounded at the apex, sharply reduced at the base into a short stalk, at first thick-walled with a narrow central lumen filled with cytoplasm, later less thick-walled and bitunicate without special apical structure, 8-spored, 17–22 × 100–140 μ. Ascospores obliquely 1–2-seriate, fusoid oblong, 2-celled, slightly constricted at the septum, the upper cell often larger and ovoid, the lower cell smaller or equal and rounded at the end, rather thick-walled, dark brown, smooth, with a large oil drop in each cell, (29–)36–48(–55) × 12–16(–19) μ. Paraphysoid filaments numerous, filiform, septate, branched, hyaline, vacuolate, 2–3 μ in diameter.

Material examined: type collection (*Sphaeria thujina*) on wood of *Thuja occidentalis,* Adirondack Mountains, New York, *C. H. Peck,* no date (the specimen was named and described in 1875); on decorticated branches and stems of *Abies balsamea,* HPFQ 18360, Duchesnay, Quebec, *D. E. Etheridge,* July 15, 1960; HPFQ 16235, Millinocket, Maine, *René Pomerleau,* Sep. 3, 1940; HPFQ 18405, Lac Mitis, Quebec, *D. E. Etheridge,* May 24, 1961.

The connection between the perithecia and the sterile cultures of the fungus obtained from bluestained wood was established by comparative studies of the latter mycelia with mycelia originating from ascospores plated on malt extract agar. A cultural description of the fungus follows:

GROWTH CHARACTERS.—Growth rather slow on malt agar, plates never completely covered after 6 weeks. Advancing zone even, narrow, brownish black (7.5 YR 3/2) [3] to dark olive brown (2.5 Y 4/2). Mat raised, zonate, cottony, plushy, with a raised button over the inoculum, brownish black, dark greyish brown at the surface. Reverse mainly deep brownish black, olive black at the margin or neutral (3/0). Odor none.

HYPHAL CHARACTERS.—*Advancing zone:* hyphae almost hyaline to light brown at the growing tip, soon deep brown, rather straight, septate, infrequently branched, 1.5–3.0 μ in diameter. *Aerial mycelium:* hyphae light brown to deep brown or deep olive brown, straight or occasionally curved, usually with long cells, sometimes closely septate, rather thick-walled, up to 6.0 μ in diameter, vacuolate or only with small or large oil drops, occasionally forming helicoidal hyphae, smooth or frequently covered with small pointed incrustations or with large rounded deep brown incrustations up to 2.0 × 1.5 μ in size (FIG. 5). No change in color with KOH and NH_4OH. Conidiophore, chlamydospores, spores, and other special structures absent.

[3] Munsell color equivalents.

Similar hyphae occur in wood, sometimes following the long axis of the tracheids, more frequently crossing the cells at right angles to form appressorium-like structures against the walls which the hyphae then penetrate with fine, constricted filaments. The bore-holes are not enlarged as in decay fungi (FIG. 6).

RESULTS

Temperature.—The mean daily growth rate of 5 isolates of *K. thujina* at different temperatures is shown in FIG. 7. The optimal temperature for growth on malt agar occurred between 22° and 28° C for 5 isolates

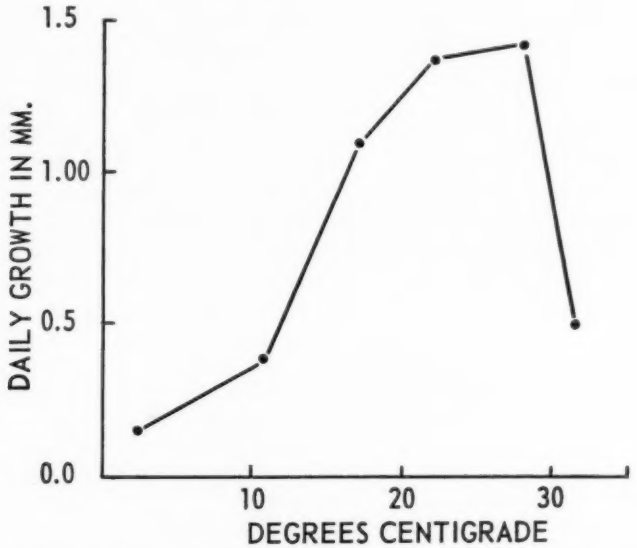


FIG. 7. Daily radial growth of colonies of *Kirschsteiniella thujina* on 2.5% malt agar for 14 days at various temperatures. Average of five isolates.

studied. Growth of all isolates was considerably reduced at 2.5° and 32° C and it ceased altogether at 36° C, although the inoculum could be revived after two weeks at this high temperature.

Moisture.—The minimal wood moisture content for growth of *K. thujina* occurred at 23.5% (o.d.w. basis) in test blocks of balsam fir heartwood incubated at room temperature (25° C) (TABLE II). Negative results obtained by visual inspection were confirmed by failure to reisolate *K. thujina* from these test blocks.

Relationship with Decay.—Because of its frequent occurrence with decay in living trees and its possible effect on the decaying activity of wood-destroying fungi, *K. thujina* was studied in combination with *Stereum sanguinolentum* (Alb. & Schw. ex Fr.) Fr. on wood and on malt agar. For the tests on wood, six cultures each of isolates HPFQ 292 and 126-C-2 of *K. thujina* were first grown on small blocks of balsam fir heartwood in screw-top jars in a humidity chamber, according to a method described by Etheridge and Morin (**5**). The initial moisture content of the test blocks was 50% (o.d.w. basis). Eleven additional jars with blocks were prepared in the same way but were not inoculated. After two months, the 23 blocks were removed aseptically from the jars, each was then wrapped in sterile aluminium foil, and the mycelia in three blocks of each series infected with bluestain were killed by steaming for 20 minutes. The blocks were weighed, the moisture content

TABLE II

MINIMAL WOOD MOISTURE CONTENT FOR GROWTH OF K. THUJINA

Moisture content of test block (% o.d.w.)	Results	Duration of test (days)
12–18	No growth	40
22.0	No growth	40
22.1	No growth	40
22.5	No growth	40
23.5*	Growth	26
24.8	Growth	22
25.2	Growth	26
28.5	Growth	13
31.8	Growth	13
31.9	Growth	13

* Minimal moisture content for growth.

readjusted to 50%, then inoculated with *S. sanguinolentum*, as shown in TABLE III, and incubated in a saturated atmosphere for a further 3 months.

The results in TABLE III indicate that, under the conditions of the experiment, bluestained wood containing living mycelia of *K. thujina* was not attacked by *S. sanguinolentum*. Where *K. thujina* was absent, and in blocks which were bluestained but where the mycelia of the fungus had been killed by steam, the wood was stained reddish brown, indicating that incipient decay of *S. sanguinolentum* had occurred. There was no measurable loss in weight associated either with the bluestain after 5 months or with incipient decay of *S. sanguinolentum* after 3 months. After 2 months, the bluestain fungus had developed extensively on the surfaces of the test blocks and by the end of the experiment the interior of the wood was stained uniformly blue. The incipient decay of *S. san-*

guinolentum had developed extensively in 3 of the 6 steamed blocks, but occurred only in patches and was confined more-or-less to the surfaces of the blocks where the bluestain was absent. The moisture level of the blocks had fallen from 50% to about 36% during the first 2 months of incubation, then following readjustment of moisture levels, from 50% to about 28% during the last 3 months of incubation. The moisture levels followed essentially the same trend in blocks which had received the different treatments. This suggested that moisture was not the factor

TABLE III

EFFECT OF K. THUJINA ON SUBSEQUENT INFECTION OF BALSAM FIR HEARTWOOD BY STEREUM SANGUINOLENTUM*

Treatment of test blocks	No. of replicates	Staining reaction of wood**		Moisture content of wood	
		After 2 months	After 5 months	After 2 months	After 5 months
Preinfected with *K. thujina* (126-C-2):					
Mycelia killed, *S. sanguinolentum* introduced.	3	blue	reddish brown; blue	33.9	28.0
Mycelia living, *S. sanguinolentum* introduced.	3	blue	blue	38.0	29.9
Preinfected with *K. thujina* (HPFQ292):					
Mycelia killed, *S. sanguinolentum* introduced.	3	blue	reddish brown; blue	33.8	29.2
Mycelia living, *S. sanguinolentum* introduced.	3	blue	blue	37.7	28.7
Not infected with *K. thujina*:					
After 2 mos. *S. sanguinolentum* introduced.	6	no	reddish brown	37.8	29.9
Uninoculated controls	5	no	no	36.9	28.3

* Blocks exposed to *S. sanguinolentum* for 3 months.

** The presence of bluestain indicates successful infection by *K. thujina*. A reddish brown stain indicates incipient decay by *S. sanguinolentum*.

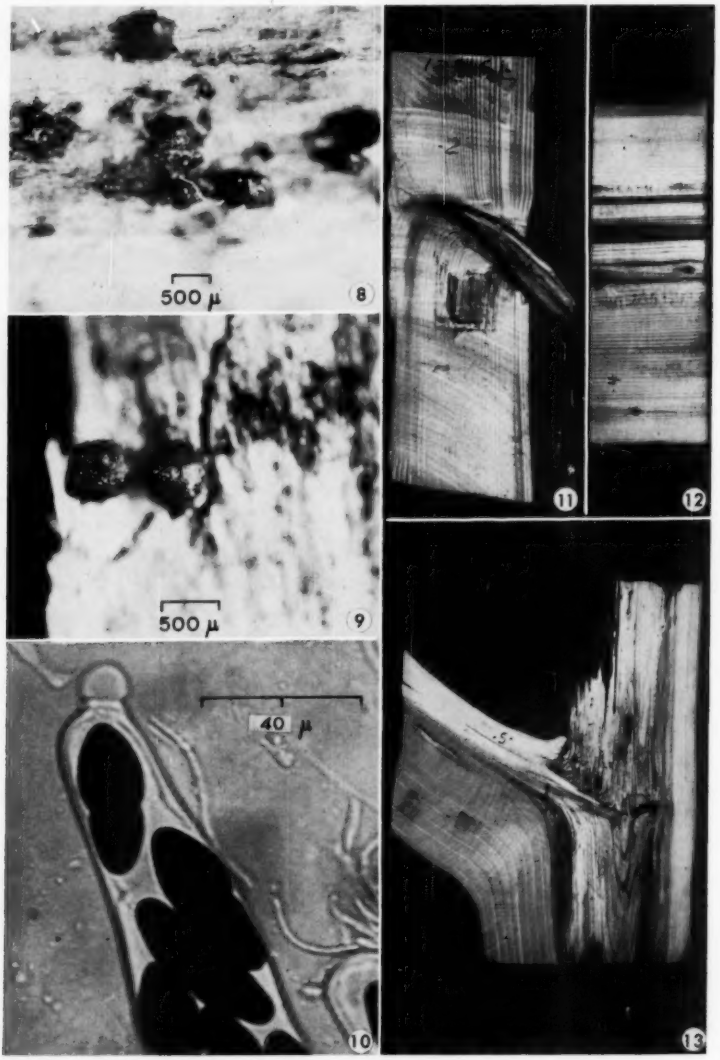
which prevented the establishment of *S. sanguinolentum* in the blocks containing living mycelia of *K. thujina*.

The interaction between *K. thujina* and *S. sanguinolentum* was tested on malt agar as follows: an agar plug from actively growing plate cultures of two different isolates of *A. thujina* was placed on the medium at the centre of a Petri dish and a similarly prepared inoculum with *S. sanguinolentum* was placed at one side of the plate. Observations were made on the mutual effect of the growth of the two fungi for 6 weeks following inoculation of the plates, but no interaction occurred

which would indicate antagonism between the fungi. No inhibition zones were formed between the colonies; after 6 weeks, one isolate of *K. thujina* was overgrown by *S. sanguinolentum* but the normal radial development of the colonies appeared to be unaffected.

Occurrence of Bluestain in Nature.—Although there are several records of bluestain occurring in balsam fir, there are little quantitative data on this defect and still less is known of its mode of entry and possible relationship with decay. Kaufert (7), in 1935, reported that 10% of overmature balsam fir in the Lake States had bluestain in the heartwood which in some cases was found around the margins of heartrot. Many dead branch stubs and overgrown knots in the trees were stained, indicating that the stain had entered into the heartwood through the avenue of dead branches. On the other hand, during the course of decay studies in mature balsam fir in Quebec, bluestain was found mainly associated with heartrot and occurred only rarely in the heartwood of trees where decay was absent. For example, of 800 balsam firs that were critically examined for decay, 321 contained trunk and basal rot infections of which 17% were associated with bluestain. The occurrence of bluestain was about equally distributed among the two kinds of rot and had presumably entered the heartwood by means of the same infection courts as had the decay fungi. In this survey, most of basal decays could be traced to root infections, whereas trunk decays were mainly associated with branches, broken tops, and various types of bark wounds. In only two instances was bluestain found in trees containing no trace of rot. The heartwood of another tree was bluestained to a height of 15 feet, which could be traced to a basal infection associated with *Corticium galactinum* (Fr.) Burt that had stopped at 3 feet. During the present investigation it was possible to obtain more information on the avenues of entry of *K. thujina* in balsam fir. A total of 880 potential infection courts were examined: 650 were branches, 171 were broken tops, and 59 comprised miscellaneous stem injuries. Cultures of *K. thujina* were obtained from 361, or 55.5%, of the branch bases (see FIG. 11); 14, or 8.2%, of the broken tops (see FIG. 13) and only 4, or 6.8%, of the stem injuries. *K. thujina* was also isolated from the roots of young balsam fir in Quebec (personal communication from Mr. E. Smerlis of this Laboratory), but no quantitative data are yet available on its frequency of occurrence in roots.

Because of its frequent occurrence with heartrot, it was of some interest to determine when *K. thujina* became established in the different infection courts. Consequently, the occurrence of *K. thujina* was determined in relation to the time when death or injury had produced the



FIGS. 8–13. *Kirschsteiniella thujina*. 8. Perithecia on decorticated wood (Peck's type collection). 9. Perithecia on wood (Quebec collection). 10. Bitunicate ascus and paraphysoid filaments. 11. Typical bluestained branch base of balsam fir from which *K. thujina* was obtained in pure culture. 12. Heart bluestain of balsam fir caused by *K. thujina* showing the characteristic short dark lines running at right angles to the wood elements. 13. Bluestain caused by *K. thujina* associated with *Stereum sanguinolentum* decay which has entered by a broken top.

potential point of entry. This study showed that bluestain was absent from the basal portion of branches for 7 years following their death and then occurred consistently and in increasing frequency in branches that had been dead for periods from 8 to 50 years. In contrast, the bluestain fungus was isolated from broken tops as early as one year following the estimated year of occurrence of the damage and was present fairly consistently in tops that had been broken for periods up to 11 years. The fungus was found only rarely associated with stem wounds at irregular intervals after the injury had occurred.

K. thujina, by far the most common branch fungus, was responsible for 71.9% of the total number of branch infections. The most frequently associated fungus was *Retinocyclus abietis* (Crouan) Groves & Wells which accounted for 48% of the successful isolations. The latter fungus, which occurred chiefly in resin-soaked tissues and in buried bark around branch bases, was isolated from branch wood as early as one year after mortality. Cultures of several other species of fungi were obtained relatively infrequently from branches only after the seventh year, but none of these was responsible for more than 7% of the infections. Decay fungi, including *S. sanguinolentum,* accounted for only 2.4% of the total branch infections. In contrast, almost 60% of the top infections were ascribed to decay fungi, most of these to *S. sanguinolentum,* while infections by *K. thujina* accounted for only 8.7% of the total.

It was noted that branches were generally resistant to bluestain and infection by other fungi for approximately 7 years following their death. A study of the moisture relations of dead and dying branches showed that the moisture content of branch bases was fairly constant at about 40% (o.d.w. basis) over the period of 6 years following their death. During the next six-year period there was a sharp drop in moisture content to 29% (about fibre saturation level) which was maintained at about this level until the branch was covered by the radial growth of the stem. It is not known whether this sharp reduction in the moisture content represents the actual death of the tissues in the buried portion of the branch or is due to physical and mechanical exclusion of the internal water supply. It suggests, however, that moisture could be an important factor in determining the successional sequence of fungi in the different infection courts.

DISCUSSION

It is clear from these studies that *K. thujina* is one of the most commonly occurring fungi in dead branches of balsam fir. The chief interest

from a pathological standpoint, however, is to be derived from the bluestain it causes and which occurs frequently in the heartwood of living trees. The stain is unusual in several respects. While most fungal stains occur independently in the sapwood of dead and dying trees, this bluestain develops in the heartwood of living trees and usually occurs in association with heartrot fungi. Unlike the mycelium of typical stain fungi, which is concentrated in the rays and wood parenchyma, *K. thujina* develops in tracheids and other wood cells, producing hyphae which characteristically pass at right angles through the walls by means of minute bore holes. This provides a useful diagnostic feature of the balsam bluestain because it can be easily recognized by the presence of numerous, short, dark lines running at right angles to the grain on tangential and radial surfaces of the wood (FIG. 12).

Whether *K. thujina* gains entry to the heartwood only as a result of its association with decay fungi or is an independent invader has not been definitely established. However, the relatively few instances where bluestain has not been found associated with heartrot suggests that its establishment in the heartwood depends, to a large extent, on the prior or simultaneous entry of decay fungi. This is borne out by the large number of bluestain infections that have been found confined to the basal portion of branches which contained no evidence of decay. Under these circumstances, penetration of the stem by bluestain appears to stop after reaching a point from 1 to 2 inches along the buried portion of branch axes. On the other hand, the occurrence of *K. thujina* with decay organisms in broken branches, and other potential points of entry, indicates that it may begin its association with decay fungi relatively early in the succession; it may then continue this relationship until the sapwood is penetrated and conditions favorable for its independent development recur.

The tendency for the stain to occur around the border of heartwood columns and to extend for appreciable distances in advance of them suggests that this may be the case. If this reasoning is correct, and the presence of *K. thujina* can arrest the decaying activity of wood-destroying fungi, as was demonstrated by laboratory studies, it is possible that bluestain may furnish an effective barrier against further development of established decay infections in living trees. Further laboratory studies are being undertaken to elucidate this relationship and to determine the basis for the apparent retardation of decay by *K. thujina*. The effect on *K. thujina* of wood previously infected by decay fungi would be a valuable corollary to such experiments.

SUMMARY

A previously reported and recently identified bluestain occurring in the heartwood of living balsam fir has been ascribed to *Kirschsteiniella thujina* (Peck) comb. nov. The morphological and cultural characters of the fungus are described and its taxonomy discussed. On malt agar, the fungus produces abundant sterile, dark grey, olivaceous aerial mycelium; the reverse of the culture is black. Growth is very slow. The optimum temperature is approximately 28° C; growth is considerably reduced at 2.5° and 32° C and it ceases altogether at 36° C; the inoculum is not killed after two weeks at the high temperature. The minimal moisture content for growth of *K. thujina* in wood occurs at 23.5% (o.d.w. basis). Bluestained wood can be recognized by the presence of dark mycelium running at right angles to the grain on both the tangential and radial planes. Unlike most fungal stains, it develops mainly in the tracheids and wood parenchyma and not in the rays. The stain is prevalent in most dead branches and rarely penetrates beyond the branch base unless decay is present. Extensive columns of bluestain which are usually traceable to the margins of heartrot may occur in the heartwood. In Quebec, bluestain has been found associated with about 17% of the butt and trunk rot infections in balsam fir. Laboratory tests have shown that *K. thujina* can inhibit decay by *Stereum sanguinolentum*, but whether it can retard the development of decay in living trees has not been determined.

ACKNOWLEDGMENTS

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STRUCTURE, GERMINATION, AND PHYSIOLOGY OF MICROSCLEROTIA OF VERTICILLIUM ALBO-ATRUM

R. S. Gordee [1] and C. L. Porter [2]

(with 9 figures)

Since the discovery of *Verticillium* wilt of peppermint in 1926, this disease has been recognized as a limiting factor in mint growing in the United States. The possibility of further decrease in peppermint oil production is a serious problem.

Control is difficult because (a) the male plant of *Mentha piperita* L. is essentially sterile, thus precluding the development of satisfactory resistant varieties, and (b) the persistence of the microsclerotia in the soil. Becker (**1**) and Thomas (**21**) concluded that the mycelium is transformed into chains of chlamydospore-like structures which coalesce, bud or branch. These cells develop thicker cell walls and become darkly pigmented.

The relationships of *Verticillium albo-atrum* Reinke & Berth. to *Verticillium* wilt of peppermint have been studied by Green (**7**), Horner (**8**), Johnson (**10**), and Luck (**13**), but comparatively few studies can be found concerning microsclerotia. Reasons for the sparse literature on this subject are that (a) microsclerotia are difficult to study on account of their durable structure and dark pigmentation, and (b) the difficulty in obtaining microsclerotia from culture free of conidia and hyphae.

Van den Ende (**23**) stated that it was the trapped conidia and attached hyphae of the microsclerotia that germinated. Pyke (**15**) demonstrated by means of heat and desiccation that germination could not be attributed to adhering conidia or mycelium. Using a chlorox decolorizing reaction, he found that germ tubes appeared to arise from cells dissimilar to the thick-walled melanized cells, and stated that these thinner-walled, conidium-like cells were responsible for the germination of microsclerotia. But, questions persist concerning the origin and nature of these heterogeneous cells.

Once it became established that microsclerotia were the primary

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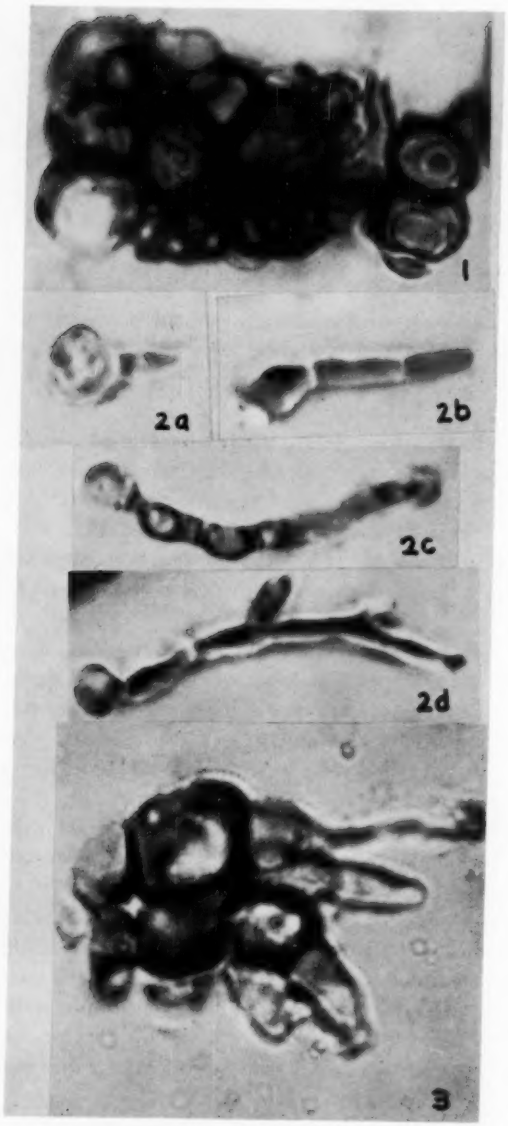

FIGS. 1–3. Microsclerotia of *Verticillium albo-atrum* (× 970). FIG. 1. A complex of typical thick-walled, melanized cells. FIG. 2. Stages of germinating thin-walled hyaline cells. FIG. 3. Two-micron section of a germinated microsclerotium showing the attachment of thin-walled hyaline cells to thick-walled melanized cells.

source of infection, intensive studies were begun hoping that some structural or metabolic weakness in them would be found that could serve as a point of attack and lead to an eventual control of this disease. The present investigation is directed along these lines and involves primarily, studies to gain further insight into the structure, germination, and basic physiology of microsclerotia.

MATERIALS AND METHODS

Microsclerotia of *Verticillium albo-atrum* (Purdue Isolate 25A) were isolated by the homogenizer-screen method of Gordee (**6**). This procedure consistently yielded clean, viable, and uniform-sized microsclerotia.

Microsclerotia were isolated from 6-week-old cultures and sonically oscillated for 20 min. Samples were placed in depression slides in humidity chambers at 25° C. Periodically, the slides were removed and phase-contrast photomicrographs were taken to record the stages of germination.

Isolated microsclerotia collected from the 200 mesh screen were placed in depression slides and allowed to germinate for 42 hrs, then treated according to the methods described by Johansen (**9**). Microtome sections 2 μ thick were stained with safranin and examined for the connection of thin-walled hyaline cells to thick-walled melanized cells.

For germination studies, microsclerotia were isolated from cultures of a designated age, and 0.15 ml quantities were placed in depression slides, which were cemented with paraffin to glass triangles. The slides were placed in humidity chambers and incubated at 25° C. Germination was recorded by observation under the low power objective of the light microscope. If a germ tube arose from a microsclerotium, this structure was regarded as having germinated. Germinated microsclerotia in ten fields on each slide were counted, and all trials were in triplicate.

Germination studies were carried out on microsclerotia to determine their viability after the sodium hypochlorite decolorizing reaction of Pyke (**15**). The effect of pH was determined by germinating microsclerotia in buffers at various hydrogen ion concentrations. The buffers were prepared according to the methods of Colowick and Kaplan (**3**). Concentrations of 10, 50, 100, and 500 ppm of cadmium were prepared from $CdCl_2 \cdot 2\frac{1}{2}H_2O$. Solutions of each cadmium concentration in 0.15 ml quantities were added to depression slides and dried at 70° C, then 0.15 ml of microsclerotia was added to the slides. The number of germinated microsclerotia was determined after 22 hrs. The effect of disodium ethylenediamine tetraacetate (di-Na EDTA) on germination

was determined in a similar manner. A standard solution of di-Na EDTA was diluted to Molar concentrations of 0.1, 0.01, 0.001, and 0.0001. In this case 0.15 ml of each concentration containing microsclerotia was added to depression slides, and germination determined.

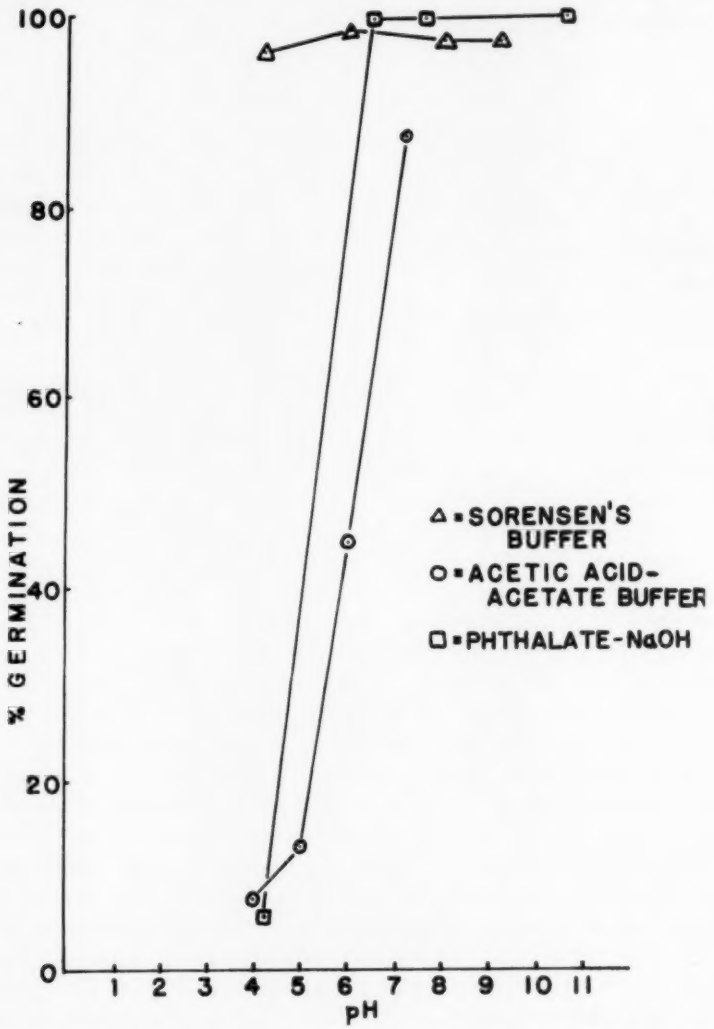

FIG. 4. The effect of buffered pH systems on the germination of microsclerotia isolated from cultures 5-months old.

The endogenous respiration of microsclerotia was determined by the gas exchange determinations of Umbreit et al. (22).

Isolated microsclerotia were centrifuged for 5 min at 6000 rpm, combined in a final volume of 50 ml, and sonically oscillated for 2 hrs. The cellular debris was removed by centrifugation at 5000 rpm for 10 min.

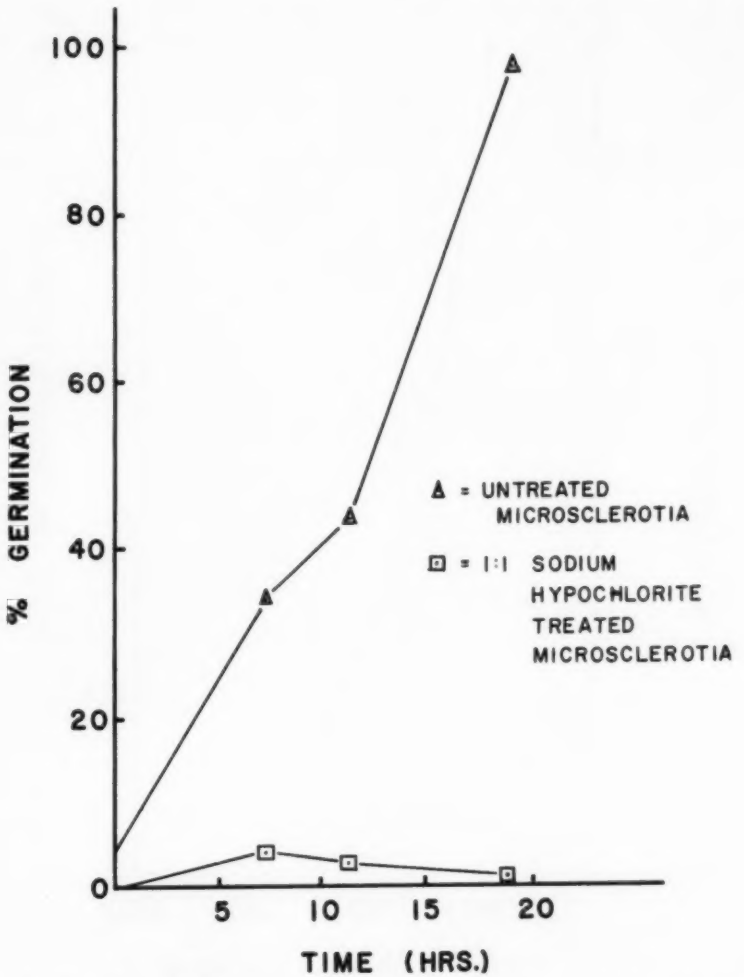

FIG. 5. The effect of 1:1 sodium hypochlorite on the germination of microsclerotia isolated from cultures 5-months old.

The dry weight of duplicate 1 ml samples of cytoplasm was determined on a microbalance.

The carbohydrate was determined by the anthrone test of Loewus (11). Protein was determined by the Folin-phenol reagent of Lowry

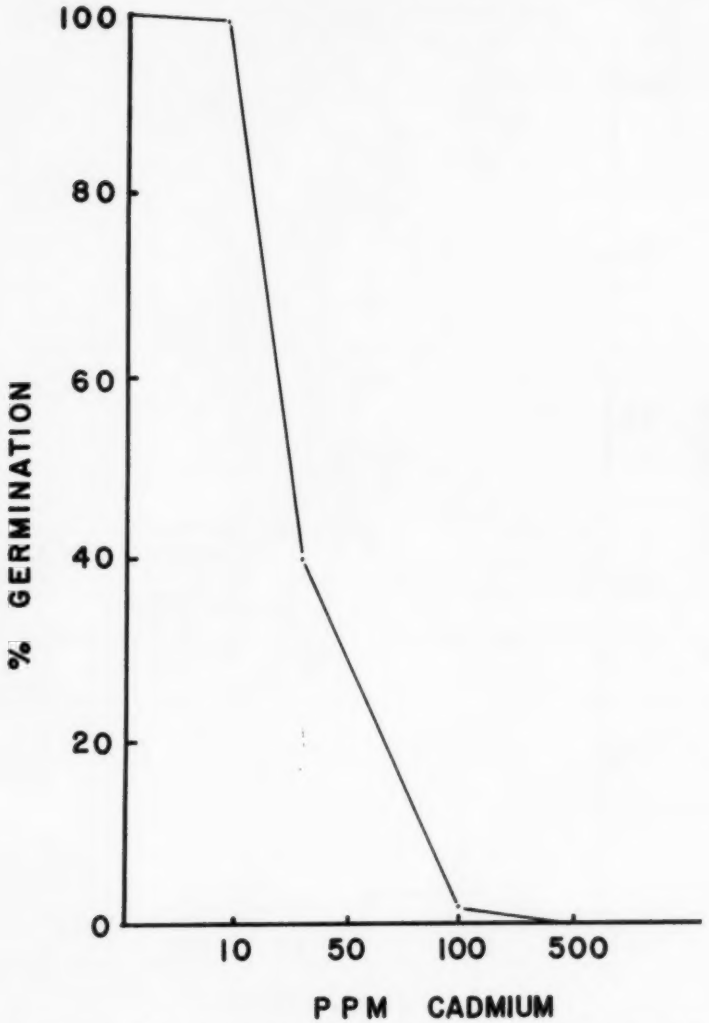

FIG. 6. The effect of cadmium on the germination of microsclerotia isolated from cultures 6-weeks old.

(12). Total lipid was obtained by extracting samples of cytoplasm dried at 35° C in a Soxhlet extractor for 24 hrs, using analytical reagent grade ether. The percent of carbohydrate, protein, and lipid was calculated on the basis of the dry weight of the cytoplasm.

EXPERIMENTAL RESULTS

The various stages of the germinating cells of microsclerotia are shown in FIG. 2. The two types of cells found were thick-walled mel-

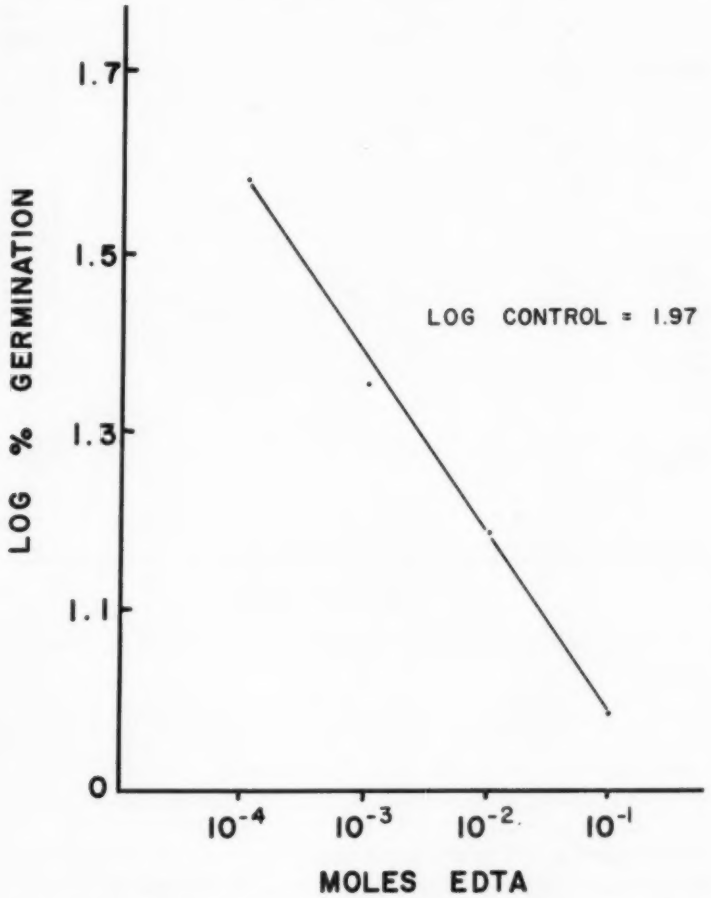

FIG. 7. The effect of di-Na EDTA on the germination of microsclerotia isolated from cultures 5-weeks old.

anized cells (FIG. 1) and thin-walled hyaline cells. Germ tubes developed only from thin-walled hyaline cells (FIG. 2). Thin-walled hyaline cells have been observed in ungerminated, year-old microsclerotia.

Microtome sections of germinated microsclerotia revealed that the thin-walled hyaline cells were attached to thick-walled melanized cells (FIG. 3). Also, the thin-walled hyaline cells were not found in all parts of the microsclerotium.

Morphologically, there was a great difference between thick-walled melanized cells and thin-walled hyaline cells. The dimensions of the

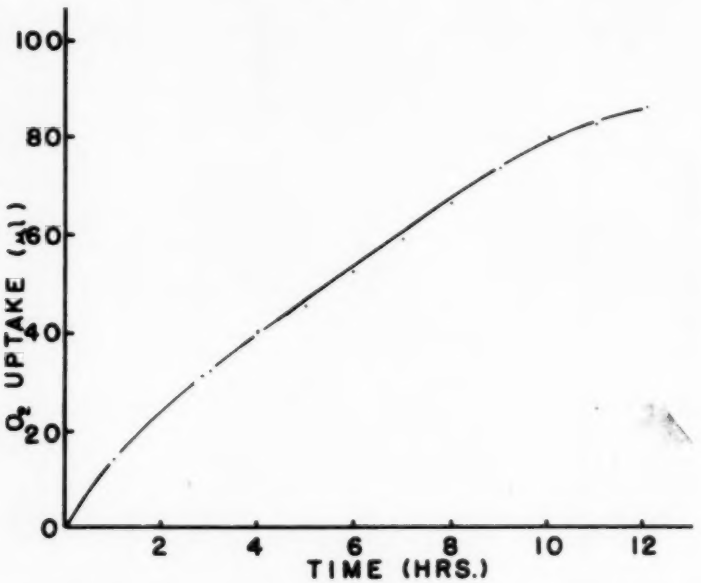

FIG. 8. The oxygen uptake by microsclerotia isolated from cultures 6-weeks old.

thin-walled hyaline cells varied from 6–10.8 × 4.8–6.0 µ, and thick-walled melanized cells varied from 6–14.4 × 6–12 µ. According to Gilman (**5**), the dimensions of the conidia of *Verticillium albo-atrum* are 5–12 × 3 µ. Thin-walled hyaline cells did not exhibit the typical ellipsoid shape of conidia and, as a result, generally had a greater width.

The results of the germination studies are summarized, FIGS. 4–7. Both pH and buffer systems affected the germination rate (FIG. 4). The sodium hypochlorite treatment inhibited germination (FIG. 5). Cadmium at 10 and 50 ppm did not arrest germination, but 100 and 500

ppm were inhibitory (FIG. 6). Di-Na EDTA at concentrations as low as 10^{-4} Molar exhibited inhibitory effects (FIG. 7).

In preliminary respiration studies of microsclerotia a total of 85.5 μl of oxygen was taken up (FIG. 8) and 46.0 μl of carbon dioxide evolved (FIG. 9) after 12 hrs of respiration. The RQ was 0.54 with a Q_{O_2} of 0.32 and Q_{CO_2} of 0.20.

The analysis of the cytoplasm of microsclerotia revealed that carbohydrate was the dominant constituent of dried cytoplasm. The protein

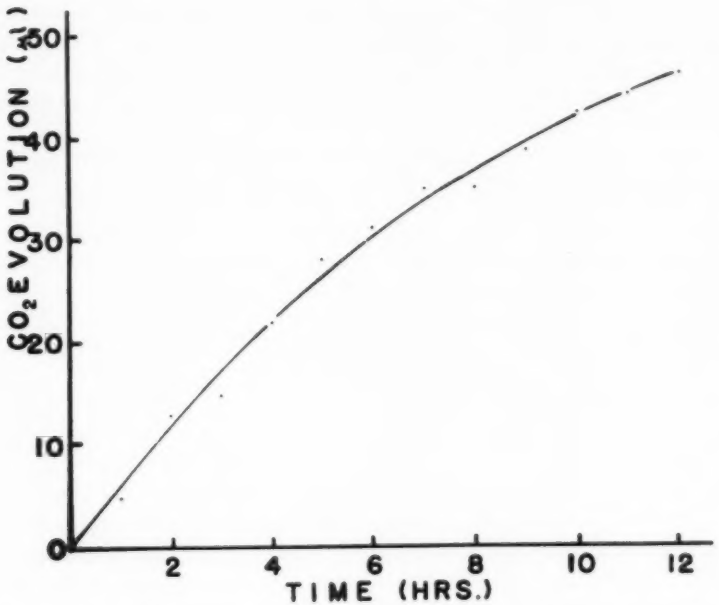

FIG. 9. The carbon dioxide evolution by microsclerotia isolated from cultures 6-weeks old.

content was fairly constant. The lipid content was intermediate between carbohydrate and protein (TABLE I).

DISCUSSION

The origin of thin-walled hyaline cells within the microsclerotium is obscure. They were not present in all parts of the microsclerotium. Microtome sections demonstrated that thin-walled hyaline cells always were attached to thick-walled melanized cells.

The sodium hypochlorite decolorizing reaction of Pyke (**15**) is excellent for studying microsclerotia *in situ*, but after such treatment these structures were not viable.

Microsclerotia exhibited a germination rate above 90% at pH 4.2–9.2 in Sorensen's buffer, demonstrating that the constituents of the buffer favored germination over a wide pH range. The acetic acid-acetate buffer limited germination to 10% at pH 4–5. Between pH 5–7.2 germination increased to 87%. Tend and Leopold (**20**) have shown acetic acid to inhibit the utilization of anions in fungus spores. The inhibition is caused by the alteration of permeability with a subsequent decrease of anions inside the cell. Neutralization reverses this effect. The mechanism of inhibition of the phthalate-sodium hydroxide buffer may be similar to that of the acetic acid-acetate buffer, as indicated by the rise in the rate of germination with increase in pH.

At 10 ppm, cadmium had little effect on germination. Fifty ppm inhibited germination 60% and at 100 and 500 ppm the inhibition was 100%. The ability to withstand 50 ppm demonstrated the ruggedness of these structures. The mechanism of metal inhibition appears to be one of adsorption of metal ions that interfere with permeability. Somers (**16**), Mueller and Biedermann (**14**), and Sussman and Lowry (**19**) presented evidence to support the hypothesis that the initial uptake of cations is on the cell surface of strongly negative binding sites.

TABLE I

AN ANALYSIS OF THE CYTOPLASM OF THREE AGE GROUPS OF MICROSCLEROTIA

Age of microsclerotia (weeks)	Dry wt. of cytoplasm (g/ml)	% Carbohydrate	% Protein	% Lipid	Total %
44	5.98×10^{-4}	58.5	8.2	21.7	88.4
9	5.81×10^{-4}	77.7	6.2	9.2	93.1
4	8.85×10^{-4}	80.0	5.6	6.3	91.9

Di-Na EDTA, at concentrations as low as 10^{-4} M, was an effective inhibitor. The inhibition is probably a result of this compound chelating essential minor elements.

An RQ of 0.54 indicates a low endogenous respiration rate, but many fungus and bacterial spores have a low respiration rate. Sussman (**17**, **18**) found that the Q_{O_2} of *Neurospora tetrasperma* was 0.54 after 290–350 min, and that 9-month-old ascospores took up 2 μl of oxygen per mg of dry weight.

Luck (**13**) found that after 320 hrs, a maximum of 1800 μl of oxygen was taken up by *Verticillium albo-atrum*. At this time microsclerotial

initials were observed, and from then on until the microsclerotia were fully developed, the oxygen uptake dropped to 750 μl after 380 hrs.

Cochrane (2) stated that lipids provide energy for germination of spores not requiring exogenous carbohydrate. However, this may not be entirely true of microsclerotia. The present studies indicate that carbohydrate is the predominant constituent of dried cytoplasm. Ergle (4) found the sclerotia of *Phymatotrichum omnivorum* to contain 36.7% glycogen. We made no attempt to separate the two cell types, and it could be that the lipid content of thin-walled hyaline cells is higher than that of the thick-walled melanized cells. Mitochondria which are deemed the site of metabolic conversions within the cell, were observed in sonically oscillated microsclerotia. As microsclerotia age, there was a conversion of carbohydrate to lipid.

SUMMARY

Microsclerotia are composed of thick-walled melanized cells and thin-walled hyaline cells. Germination of sonically oscillated microsclerotia revealed that germ tubes developed from thin-walled hyaline cells.

Thin-walled hyaline cells were not found in all parts of the microsclerotium, but where present were connected to thick-walled melanized cells.

There was a wide variation in germination in various buffers. Sodium hypochlorite-treated microsclerotia were not viable. Cadmium at 100 and 500 ppm inhibited germination completely, but 10 and 50 ppm were not significantly inhibitory. Disodium ethylenediamine tetraacetate at 10^{-4} M was inhibitory.

An RQ of 0.54 indicates that microsclerotia have a low endogenous respiration rate.

An analysis of the cytoplasm of microsclerotia revealed that carbohydrate is the predominant constituent of dried cytoplasm of the microsclerotia. Protein was a fairly constant constituent. As microsclerotia age, a conversion of carbohydrate to lipid occurs.

ACKNOWLEDGMENTS

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PARASITISM OF OLPIDIOPSIS INCRASSATA ON MEMBERS OF THE SAPROLEGNIACEAE. I. HOST RANGE AND EFFECTS OF LIGHT, TEMPERATURE, AND STAGE OF HOST ON INFECTIVITY

MIRIAM K. SLIFKIN [1, 2]

To get a better understanding of the physiological relationship between host and parasite it is important to determine just what effects the environment and nutrition have upon these organisms. In this study an effort was made to determine the host range in the family Saprolegniaceae of *Olpidiopsis incrassata* Cornu under controlled laboratory conditions. Tests were made to determine the effects of light, temperature, and the effect the stage of the host has on its ability to resist the parasite.

Both Shanor (**1940**) and Sparrow (**1960**) recognize *O. incrassata* as a member of the genus *Olpidiopsis* that has colorless warts and an adjacent empty cell. This species, according to Shanor, is parasitic on members of the genus *Saprolegnia;* however, in the original description Cornu (**1872**) reported *O. incrassata* as being parasitic on *Achyla racemosa.* In this study *O. incrassata* was found parasitizing *Saprolegnia ferax.* In the host range studies *O. incrassata* was not capable of parasitizing *A. racemosa.* These results agree with those of Shanor (**1940**). As indicated by Shanor, Cornu did not use a pure culture and it is possible that Cornu's identification was in error. While this may be so, I would like to offer a more intriguing possibility.

In an earlier work, Slifkin and Gutowsky (**1958**) showed that in the slime mold *Dictyostelium discoideum* Raper and the bacteria it feeds upon much depended upon the medium on which the bacteria grew. The relationship between the slime mold and the bacteria could be drastically altered by a change in nutrition. Butler (**1957**) demonstrated a similar situation with *Rhizoctonia solani* Kühn. He showed that the nature of a linkage separating two glucose residues could affect the

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ability of *R. solani* to parasitize *Mucor recurvus* Butler. Berry (**1959**) and Shigo (**1960**) were both able to show that in some instances not only nutrition, but also temperature and light could affect the degree of parasitism. It is possible that such is the case here. Unfortunately, the conditions under which Cornu discovered his organisms were not described to any great extent, and, therefore, they could not be duplicated here to test this thesis.

Although many studies on the genus *Olpidiopsis* have appeared in the literature, these were mostly taxonomical or morphological. Comparatively little of the physiology and almost nothing of the external conditions by which *O. incrassata* and its hosts are limited is known. An effort was made in this study to establish some environmental limits and to give an insight into the host-parasitic relationship between *O. incrassata* and the Saprolegniaceae.

PROCEDURE

With the exceptions of one strain each of *Achyla colorata, A. apiculata,* and *A. treleaseana,* which were obtained from Dr. Alma W. Barksdale, the cultures used in this study were collected in nature. Most of them were found in or near Chapel Hill, North Carolina, by the author and her family. Others were found near Linville Falls, N. C., Birmingham, Alabama, and Atlanta, Georgia. *A. bisexualis* (male and female strains) and *Saprolegnia Kauffmaniana* were found in Prince George County, Maryland, by Dr. E. V. Palmatier.

The host cultures were purified by the following method: The original culture was washed several times and the zoosporangia allowed to discharge. Zoospores were picked up with a capillary pipette and placed on agar. These were rolled on the agar,[3] single spores were placed on corn meal agar and allowed to germinate. After germination a portion of the mycelium that had grown away from any bacteria was cut off and placed on new corn meal agar. All stock host cultures used were maintained on corn meal agar slants. When a culture was used in an experiment, it was transferred to a sterile petri dish containing a hemp seed in sterile distilled water. The original host species, *S. ferax,* on which *O. incrassata* was found, was isolated by a method suggested by Shanor (**1939**). A young infected hypha was washed and then placed on a dish of corn meal agar. The parasite did not grow, but the host grew

[3] Rolling is used here to describe the process by which a spore or a sporangium is partly freed of bacteria. The spore while being observed under the stereomicroscope is manually pushed over and through the agar until the excessive bacteria have been removed.

very well. After the mycelium of the host had grown away from the bacteria, a portion was cut and placed on hemp seed in sterile distilled water. This was recultured on hemp seed and used as the host of the parasite.

Olpidiopsis incrassata was purified by the following method: The original culture was washed thoroughly with distilled water, a single sporangium was cut and rolled on agar, and washed with sterile distilled water until it discharged. The discharged zoospores were pipetted into a 24-hour host culture on hemp seed. After two days the new culture was washed again and a zoosporangium was cut and rolled on agar. Again discharged zoospores were pipetted into a 24-hour host culture. This procedure was repeated until no bacteria could be detected by phase microscopy or on a nutrient culture medium. Stock cultures of the parasite were maintained by introducing an infected culture into a dish containing a 24-hour host culture on hemp seed in distilled water. Checks were made at frequent intervals to test for presence of bacteria. When contamination was detected, the purification procedure was repeated.

In the host range studies, zoospores of *O. incrassata* were introduced into 24-hour host cultures on hemp seed. If no infection occurred, a heavily parasitized culture was added to the test cultures and then examined for infection after 24 hours.

Unless otherwise stated all cultures used were tested on hemp seed in sterile distilled water and incubated at 20° C under intermittent light.

At least one isolate of each species of the family Saprolegniaceae was tested for susceptibility to *O. incrassata*. In many cases the same species was found several times; for example, *A. flagellata* was collected 17 times. Two isolates that were found in different habitats and at different seasons of the year were tested to determine whether there was any variation in susceptibility.

The host species of all genera except *Achlya* were classified according to Coker and Matthews (**1937**). The species of *Achlya* were classified according to Johnson (**1956**).

RESULTS

Host Range: TABLE I shows that *O. incrassata* was parasitic on all species of *Saprolegnia*, with the exception of the imperfect form, *S. parasitica*. It was also parasitic on both species of *Isoachlya*. *Protoachlya paradoxa*, *Dictyuchus* sp., *Thraustotheca clavata*, three isolates of *Aphanomyces*, and all species of the genus *Achlya* tested proved immune to infection by *O. incrassata*.

These results confirm and add to the range reported by Shanor (1940). The habitat of the host organism and the season of collection seem to play no part in whether or not an organism is immune to *O. incrassata*. Tests were made to compare the susceptibility of like species that were collected in unlike circumstances. For example, one sample of *S. delica* was collected during the summer in a stream at Linville Falls, N. C. and another was collected in early spring from a forest soil sample in Chapel Hill, N. C. Both of these were susceptible to *O. incrassata*.

TABLE I

HOST RANGE OF OLPIDIOPSIS INCRASSATA ON MEMBERS OF THE SAPROLEGNIACEAE

Host species	Susceptibility	Host species	Susceptibility
Saprolegnia delica Coker	×	*A. treleaseana* (Humphrey) Kauffman	0
S. diclina Humphrey	×	*Isoachlya anisospora* (DeBary) Coker	×
S. ferax (Gruith) Thuret	×	*I. monolifera* (DeBary) C. H. Kauffman	×
S. Kauffmaniana Pieters	×	*I. unispora* Coker & Couch	×
S. mixta DeBary	×	*Protoachlya paradoxa* (Coker) Coker	0
S. parasitica Coker	0	*Dictyuchus sp.* (imperfect)	0
S. sp.	×	*Thraustotheca clavata* (DeBary) Humphrey	0
Achlya americana Humphrey	0	*Aphanomyces laevis* DeBary	0
A. apiculata DeBary	0	*Aph. scaber* DeBary	0
A. bisexualis Coker & A. Couch	0	*Aph. stellatus* DeBary	0
A. colorata Pringsheim	0		
A. conspicua Coker	0		
A. diffusa Harvey ex Johnson	0		
A. dubia Coker	0		
A. flagellata Coker	0		
A. inflata Coker	0		
A. klebsiana Pieters	0		
A. megasperma Humphrey	0		
A. oblongata DeBary	0		
A. prolifera C. G. Nees	0		
A. proliferoides Coker	0		
A. racemosa Hildebrand	0		

× — susceptible; 0 — not susceptible.

There were many other similar cases, but in all cases the same species showed the same reaction regardless of environment.

Optimum Stage for Infection: A stock culture on hemp seed in water was made of each species in TABLE I that became infected with *O. incrassata*. As cultures were needed, hemp seeds were added to the stock culture. To test for the age of maximum susceptibility of the host, hemp seeds were added to the stock cultures 16, 24, 48, and 72 hours before they were to be tested. The cultures were inoculated with *O. incrassata* incubated at 20°. They were examined at 24-hour intervals.

There was no noticeable difference between the species in the stage at which they were most susceptible to the parasite.

As is shown in TABLE II, after 1 day the 24-hour culture showed the greatest amount of infection. The 16-hour culture was slightly infected, and the 48- and 72-hour cultures were not infected. After 2 days the 16-hour culture was heavily infected, the 48-hour cultures slightly and the 72-hour cultures not at all. The infection on the 48-hour culture took place on the new hyphae near the hemp seed, the older hyphae were not infected. The 72-hour culture was observed every day for a week but no infection occurred.

In no case did infection occur after the initiation of zoosporangia or oogonia. These results raise three questions: (1) Does the initiation of sex cells or zoosporangia have an inhibitory effect on parasitism? (2) Does the cell wall act as a barrier to the parasite? (3) Does the actual age of the cell wall affect parasitism?

TABLE II

TEST FOR OPTIMUM AGE FOR INFECTION

Age in hours of culture when exposed to parasite	Days after exposure to parasite				
	1	2	3	4	5
16	×	××××			
24	××××	××××			
48	0	×	××	××	
72	0	0	0	0	0

Extent of infection by *Olpidiopsis incrassata*: 0 = none, × = slight, ×× = fair, ××× = good, ×××× = very good infection.

In an effort to answer these questions, the following experiments were pursued: A hemp seed culture of *S. ferax* was permitted to grow beyond the susceptible stage to produce zoosporangia and oogonia. On one side of the hemp seed the sporangia were cut off, the other side was left undisturbed. It was then inoculated with *O. incrassata* and incubated. A control was run simultaneously in which both sides of the host culture were cut. After 24 hours, the cut portion of the host culture was heavily infected with the parasite, and the uncut portion continued its development to form mature oospores. In the control, zoosporangia and oogonia were formed after 24 hours.

This experiment was repeated ten times using *S. ferax* as the host culture and was tried once on each of the other species of *Saprolegnia* with the exception of *S. parasitica*. The same results were obtained every time.

To test whether or not there is something in the mycelial wall that can inhibit infection, similar tests were made with other species of the Saprolegniaceae. Those tested were *Protoachlya paradoxa, Dictyuchus* sp., *Thraustotheca clavata, Achlya colorata, A. americana, A. flagellata, A. apiculata, A. bisexualis* (each sex separately and both together), *Aphanomyces laevis* and *S. parasitica.* These were treated in the same manner as those described above, but in no case did infection with *O. incrassata* occur. The cut portions continued development. The mycelium healed and behaved as a young mycelium producing zoosporangia, antheridia and oogonia.

When a sheet of cellophane was placed on the surface of a dish containing corn meal agar, and inoculated with *S. ferax,* the organism produced only mycelium for at least four days. After this gemmae and oogonia began to form. By this method is was possible to have mycelium that was free of reproductive structures for an extended period of time. The method was useful for testing the third question, that is, whether or not the actual age of the mycelium affected parasitism or acted as a barrier to the parasite. Twelve plates were prepared in this manner using *S. ferax.* After one day three dishes were inoculated with zoospores of *O. incrassata,* after two days another three were inoculated with the parasite and so on for four days after the inoculation of the host. Each plate was flooded with distilled water when it was inoculated with the parasite. A control containing only the host was run simultaneously.

The control began to show evidence of oogonial initials and gemmae on the fourth day. These were few in number until the fifth day, and as long as the cellophane was on the agar surface, the oogonia never became numerous. Infection was very good in the plates with hosts one and two days old. No gemmae or sporangia were found. Both 3- and 4-day-old host cultures gave good infection. Infection occurred throughout the culture and not just on the young growing tips. This again showed that infection occurred where no zoosporangia or oogonia have been initiated. It indicated that the age of the mycelium is not a factor of susceptibility but rather the stage of development seemed to be significant.

Effect of Light: Six plates containing cultures of *S. ferax* were inoculated with *O. incrassata.* Three plates were placed in light proof boxes in the dark, and the other three were put under continuous fluorescent light. In all previous experiments cultures were exposed to intermittent light.

All cultures whether placed on continuous light, complete darkness, or intermittent light became infected and grew equally well. The reac-

tions were completely normal and all stages in the life cycle of *O. incrassata* and in the host cultures were seen. It can be safely assumed that light, or the lack of it, has no effect on infection by *O. incrassata* or on the life cycle of *O. incrassata.*

Temperature Studies:

a. *Host range.*—The host range of *O. incrassata* was tested at 6°, 20°, and 30° C. The organisms listed in TABLE I were used in this experiment. Each species was tested a minimum of three times at each temperature. The procedure was the same as described earlier, except that the number of positive tests at 30° were so few that a different method had to be used. In cases where infection occurred at 20° an infected culture was added to a 24-hour culture of the same species and incubated at 30°. Where infection did not occur at 20°, as in species of *Achlya,* heavily infected cultures of a different species had to be used to supply the inoculum. The infected cultures were so heavily parasitized that no host spores were produced. This eliminated the possibility of spores from the inoculum culture growing onto the hemp seed of the test culture and causing a false positive reading.

Infection at 30° in all cases was very slight. The host range was the same at all three temperatures.

b. *Temperature range.*—This study was made to determine the maximum and minimum temperatures of both hosts and parasite.

The maximum temperature for *S. ferax* was 33°. At this level only a very thin mycelium grew. Within the mycelium dense areas of protoplasm were seen. No differentiation of any kind took place. It was unable to reproduce itself by way of zoospores or by oospores. At 33° *O. incrassata* could not parasitize *S. ferax.* The highest temperature at which parasitism occurred was 30°.

At 6°, the lowest temperature tested, the host grew slowly. As an example *Saprolegnia* sp. grew as much in one day at 20° as it did at 6° in three days. The growth of mycelium looked slightly abnormal. The oogonial initials and the mature oogonia and eggs appeared normal.

Studies were made at 10°, 15°, and 25°. Except that the reaction was slow at 10° there were no exceptional differences between the results at these temperatures and the results at 20°.

c. *Effects of 30° C.*—Growth of most of the *Saprolegnia* spp. grown at 30° was highly abnormal. The mycelium was thick and the protoplasm concentrated in spots instead of being distributed throughout the mycelium. There was excessive formation of gemmae. Few zoosporangia were formed, but these were very abnormal. None were seen to discharge. No oogonia or antheridia were observed.

At 30° the growth of *O. incrassata* was normal for a day or so, then the sporangia began to produce oil droplets and disintegrate. No resting bodies were formed at this temperature. If a new 24-hour culture of the host was added to the infected culture and left at 30°, reinfection did not take place. If after 24 hours at 30° the cultures were removed to 20°, reinfection would occur. If after 48 hours at 30° the cultures were moved to 20°, the chances of reinfection occurring were remote. Any period after that reinfection never occurred (TABLE III). When the recently infected culture was left at 30° for several days, the sporangium of the parasite degenerated and no sex cells were formed. If a culture was grown at room temperature until resting bodies were formed and then placed at 30°, no degeneration was seen.

TABLE III

EFFECT OF A TEMPERATURE OF 30° C*

Incubation temperature 24-hour periods					Type of reinfection
1	2	3	4	5	
20°	30°#	20°′	20°	20°	heavy
30°	30°#	20°′	20°	20°	heavy
30°	30°#	30°′	30°	30°	none
30°	30°#	30°	20°′	20°	very slight
30°	30°#	30°	30°	20°′	none

* In the first 24-hour period the hemp seed was exposed to an active host culture and growth occurred. At the beginning of the second 24-hour period a culture of *O. incrassata* was introduced. This is indicated by the symbol #. The symbol ′ is used to indicate that a new 24-hour host culture grown at 20° has been added to test for reinfection.

DISCUSSION

Olpidiopsis incrassata has been shown to be parasitic on all species of *Saprolegnia* tested with the exception of *S. parasitica*. It was not parasitic on any members of the genus *Achlya*. The one member of the genus *Saprolegnia* that *O. incrassata* did not parasitize was the imperfect form that does not have sex cells.

One important factor used in determining species of *Olpidiopsis*, besides the morphological characters of the resting spores, has been the host genus on which certain species were found. *O. incrassata* appears to be host specific for the genus *Saprolegnia* and the closely related genus *Isoachlya*. It would be erroneous to conclude from this that *S. parasitica* is not as closely related to other species of *Saprolegnia* as are species of *Isoachlya*. In this case it may be wiser to assume that something is missing from the imperfect form that is supplied in the perfect.

Although the parasite does not infect the reproductive stages of the host, it appears likely that there is some enzyme or other chemical that is a precursor to the reproductive stages that is also necessary for infection by *O. incrassata*. One indication of this lies in the host range studies where *S. parasitica* is not infected. Once reproductive structures are initiated and a possible "reproductive precursor" is used or changed, the particular mycelium containing this structure is immune. When the oogonia or sporangia are cut off and the organism has to revert back to the vegetative stage, it is again susceptible to the parasite.

There are other possible explanations for immunity or susceptibility: (1) The cell wall may act as a barrier to penetration. This theory has been shown to be rather weak for the host fungi studied. When the mycelium was cut, both portions of the organism were left exposed to the parasite. Only the part that could later develop reproductive bodies was infected while the parts that had the reproductive bodies already initiated were immune. *Achlya* spp. and *S. parasitica* when cut were still immune. (2) The age of the organism may act to deter parasitism. In the experiment using cellophane over corn meal agar, it was shown that as long as the initiation of sex cells could be delayed, parasitism could occur. (3) It is possible that the host organism after it reaches a certain stage in its development produces a substance that makes it resistant. While no definite tests were made to prove or disprove this, in the experiment in which the oogonia were cut off, the portions cut off were left in close proximity to the rest of the mycelium and still there was good infection by the parasite. If there was a substance produced by the oogonia that makes the organism resistant, it is very weak.

Although it is possible that each of these explanations is partly responsible for immunity or susceptibility, the indications are that the "reproductive precursor" plays the major part in susceptibility.

Barksdale (**1960**) was able to show that temperature can have a profund effect on the production of sexual organs in some species of *Achlya*. It was hoped that by using extreme temperature variations, possibly the sexual reactions of various members of the Saprolegniaceae would be changed and in turn the host range of *O. incrassata* would be changed. The host range at 6° and that at 30° were the same as that at 20°. Temperature did seem to make a difference in other respects. At 6° growth was slowed to approximately one-third that at 20°, also very few resting bodies of the parasite were formed. This agrees with studies done by Barnett and Lilly (**1958**) who showed that lower than optimum temperatures reduced the rate of growth of both *Calcarisporium parasiticum* Barnett and its host, but with temperatures as low as 10°

few spores were produced. In the present study, at 30° it was much more difficult for *O. incrassata* to infect, and once it did do so, it was incapable of producing resting bodies. Unless the sporangia were discharged rapidly they would produce large oil globules and then degenerate. Once the resting bodies were produced at a more favorable temperature, they were able to withstand 30°. Unfortunately, the author has not yet been able to germinate resting bodies to test whether or not the high temperature affected germination. There was no noticeable difference in the parasitic reactions at 10°, 15°, 20°, and 25° C.

The presence or absence of light did not produce a change in parasitic reactions.

SUMMARY

The host range of *Olpidiopsis incrassata* was tested on 32 members of the Saprolegniaceae. It was parasitic on all members of the genus *Saprolegnia*, except for *S. parasitica*, and was parasitic on *Isoachlya unispora* and *I. monolifera*. It was not able to parasitize any member of the genus *Achlya*, *Protoachlya paradoxa*, *Dictyuchus* sp., *Thraustotheca clavata*, *Aphanomyces laevis*, *A. scaber*, or *S. parasitica*.

Presence or absence of light does not affect either the growth of host or parasite.

A change in temperature does not affect susceptibility of a host to *O. incrassata*. A temperature of 6° C retards the growth of the parasite and reduces greatly the number of resting bodies that are formed.

It is difficult to infect a host at a temperature of 30° C. *O. incrassata* grown at 30° will not infect another host unless put at a lower temperature. Degeneration of the parasite will occur at 30° if left at that temperature for 24 hours. When the parasite is established at a more reasonable temperature and then placed at 30°, degeneration will not occur.

O. incrassata appears unable to infect any saprolegniaceous host after zoosporangia or oogonia have been initiated. The indications are that there is some enzyme or other chemical that is a precursor to the reproductive stages of the host that is also necessary for infection by *O. incrassata*.

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FINE STRUCTURE OF MYCOTA. 5. LOMASOMES—PREVIOUSLY UNCHARACTERIZED HYPHAL STRUCTURES [1]

ROYALL T. MOORE [2] AND JAMES H. MCALEAR [2]

(WITH 11 FIGURES)

A survey of many genera of fungi with the electron microscope has served to point up the fact that there are many structures in the cells of these organisms of currently unknown origin, function, ontogeny, or even identity. One such structure that we have found repeatedly in the Eumycota is, in this report, named and its description, occurrence, and possible function are discussed.

MATERIALS AND METHODS

The fungi, listed below, were prefixed in unbuffered aqueous 2% $KMnO_4$ for ca. 10 minutes and then fixed 3–5 hours at 4° C in buffered 2% OsO_4; subsequently they were rinsed in buffer alone, dehydrated in an ethanol series, and embedded in a methacrylate mixture. A full description of this protocol appears in the first paper in this series (Moore, R. T. 1962. Nova Hedwigia, in press). Sections were cut on a Serval Porter-Blum ultramicrotome using a Fernández-Morán diamond knife and examined in a Siemens Elmiskop I. The following are the fungi reported in this study:

PHYCOMYCETES

Syncephalastrum sp. (−mating type) (QM 6793)

ASCOMYCETES

Coryne sp.

Mollisia sp.

Neobulgaria pura (Fries) Petrak

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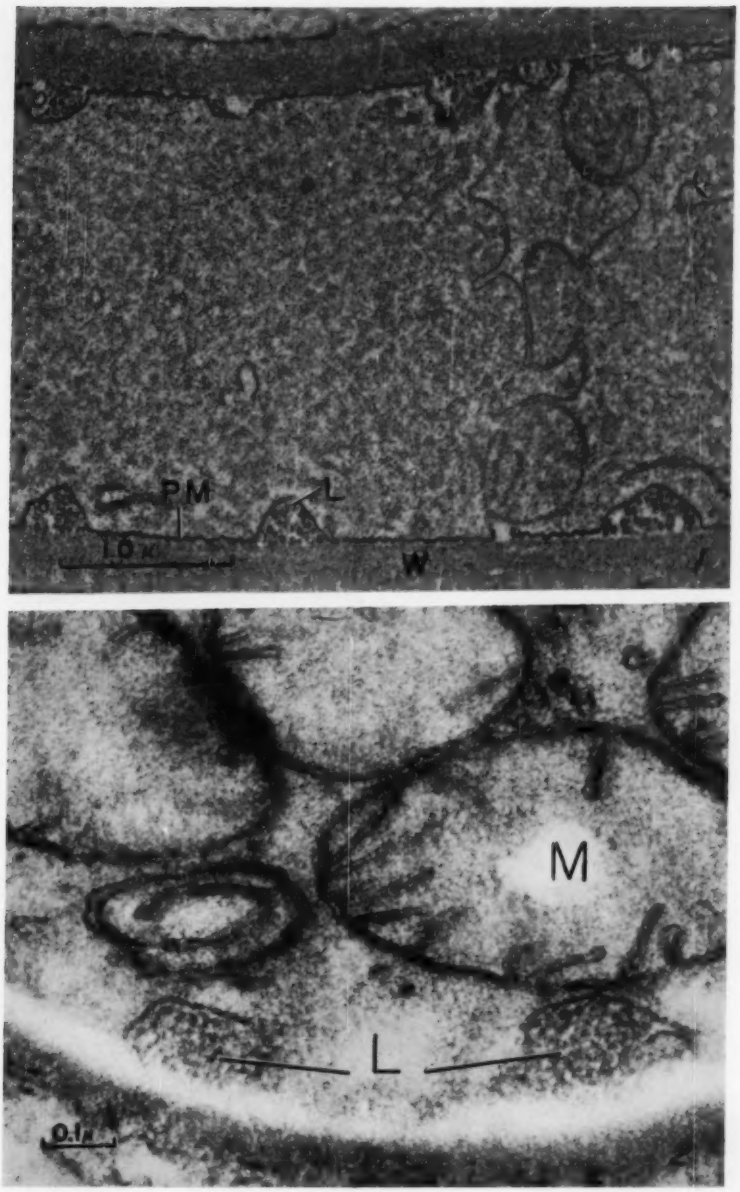
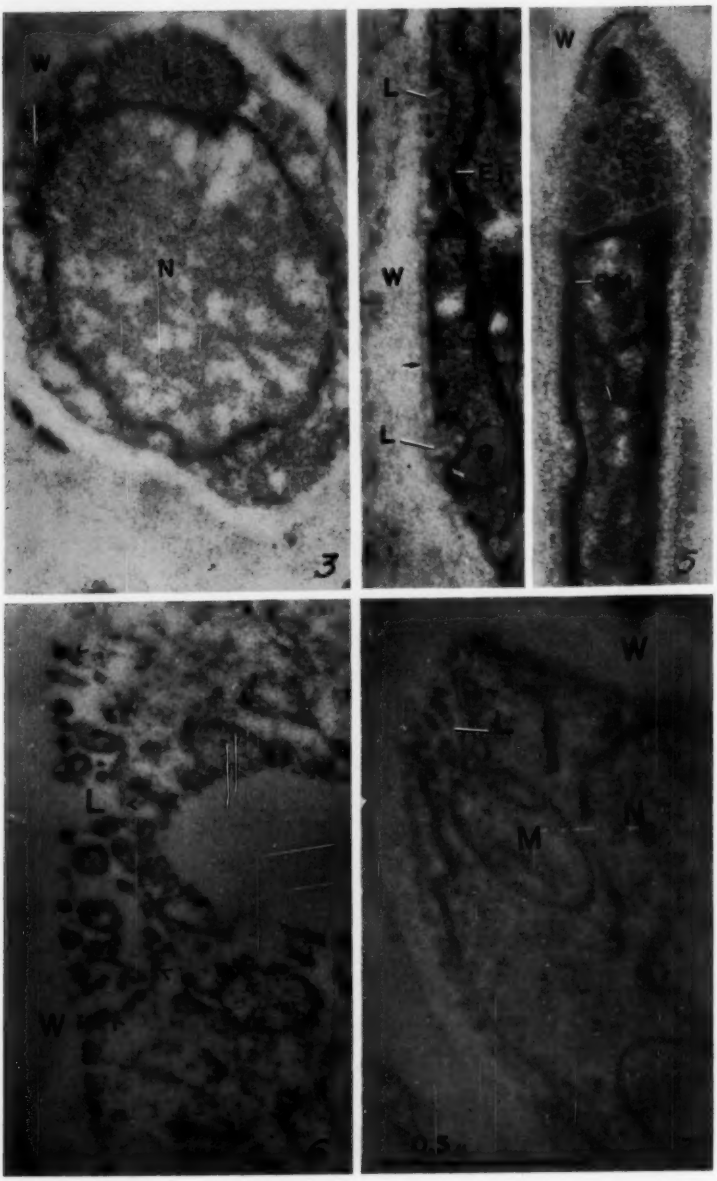


FIGS. 1, 2. Lomasomes (L) of Basidiomycetes. 1. *Schizophyllum commune*, W—hyphal wall, PM—plasma membrane (the unusual endomembrane configuration is at present not understood); × 25,000. 2. *Uromyces caladii*, portion of a haustorium, M—mitochondrion; × 100,000.



FIGS. 3–7.

BASIDIOMYCETES

Auricularia auricularis (S. F. Gray) Martin
Dacrymyces deliquescens (Bull. ex Mérat) Duby var. *minor* (Peck) Kennedy
Merulius tremellosus Schrad. ex Fries
Schizophyllum commune Fries
Uromyces caladii (Schw.) Farlow

OBSERVATIONS

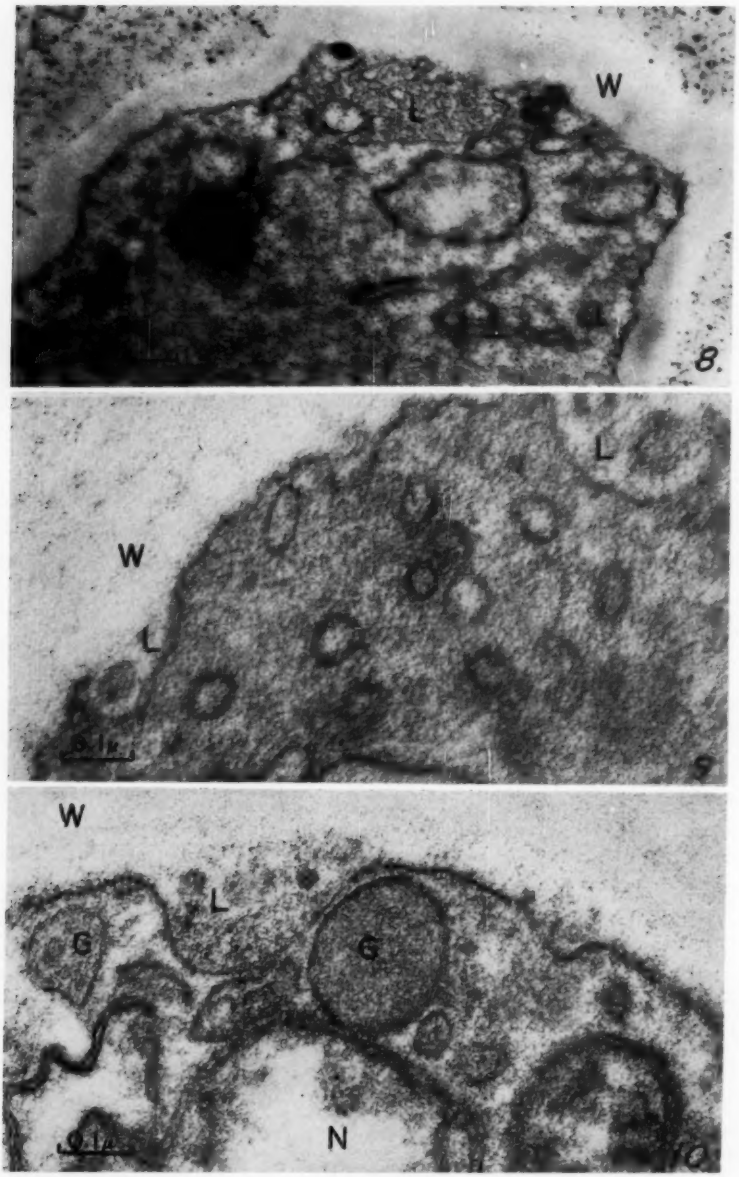
FIGS. 1–10 show portions of the hyphae of the cited fungi. In each figure may be seen a sponge-like structure (L) contiguous with the wall (W) and whose interior limits are defined by a variously sharp, dark line —the plasma membrane (PM). From its position in the cell we have chosen to call this formation the **lomasome** (λωμα—border; σωμα—body).

Lomasomes vary not only in content which appears to be granular or vesicular, or both, but also in extent and distribution along the cell wall. They do not appear to invade the cell wall but to be an addition to it and are probably dome-shaped intumescences rather than bands of material. We have observed lomasomes in about a quarter of the examined hyphae, but have thus far failed to find them in conidia, ascospores, basidiospores, or the cells from which these arise. With the possible exception of the inner ectal excipulum of *Neobulgaria pura* there does not appear to be any obvious correlation between the occurrence of lomasomes and the presence or concentration of cellular organelles. In the cells of the pseudoparenchymatous layer of *N. pura* (FIGS. 9, 10), there are, however, vesicles in the cytoplasm adjacent to the lomasomes, some of the latter of which (FIG. 9) do not appear to be highly developed.

DISCUSSION

The propinquity of vesicles and lomasomes in *Neobulgaria pura* suggests a mechanism for lomasome formation, i.e., that such vesicles

FIGS. 3–7. Lomasomes (L) of Eumycota; × 60,000. 3. *Merulius tremellosus*, W—hyphal wall, N—nucleus. 4. *Dacrymyces deliquescens* var. *minor*, W—hyphal wall, G—granule, ER—endoplasmic reticulum, (arrow)—denotes a line of demarkation between primary wall and what may be a thin secondary deposition, note that this line passes beneath the lower lomasome. 5. *Auricularia auricularis*, support hypha, W—hyphal wall, PM—plasma membrane. 6. *Syncephalastrum* sp., W—hyphal wall, (wedges)—indicate lomasome delimitation. 7. *Mollisia* sp., W—hyphal wall, M—mitochondrion, N—nucleus.



FIGS. 8–10. Lomasomes (L) of Ascomycetes. 8. *Coryne* sp., W—hyphal wall; × 30,000. 9, 10. *Neobulgaria pura*, W—hyphal wall, G—granules, N—nucleus; × 100,000.

may move out and fuse with the plasma membrane releasing substances outside the protoplast (FIG. 11) and that subsequently an electron transparent material is deposited around the ejected material. However, in as much as the function and chemical composition of lomasomes are unknown the content of these vesicles could be either deposits of secreted ergastic substances trapped between the plasma membrane and the cell wall or deposits of stored material; also, they could in some way be involved in cell wall metabolism.

There is at present no evidence for the occurrence of lomasomes in any cells except those of the Eumycota; they have not been reported in bacteria, actinomycetes, protozoa, animals or plants. The lack of evidence for the occurrence of lomasomes in the latter groups may provide

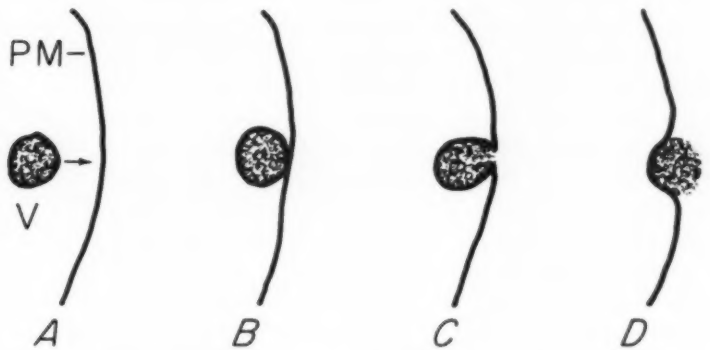


FIG. 11. Steps in the fusion of vesicle and plasma membranes. A. Vesicle (V) approaching the plasma membrane (PM). B. Contact and early fusion of the two membranes. C. The fusion point has become dissolved and the vesicle membrane is becoming incorporated into the plasma membrane resulting in the latter's enlargement. D. Later stage of incorporation begun in Stage C; note that this process results in the contents of the vesicle being released to the outside of the protoplast.

the basis for a fundamental structural characteristic that at the subcellular level could offer further support for the idea of the integrity of the Eumycota as a distinct taxonomic group. Surveys of algal structure are not as complete as those of fungal structure. Should lomasomes be found in any of the algae, they could conceivably be of great value in determining the phylogenetic origin of the Eumycota. At present we may say that lomasomes represent the only structure which we have found to occur in fungi and not in other cells.

ACKNOWLEDGMENTS

We wish to express our sincere appreciation to Dr. E. G. Simmons, Natick Quartermaster (QM) Center, for supplying the culture of *Syncephalastrum* sp. and to Prof. G. W. Martin for his kindness in identifying material of *Dacrymyces deliquescens* var. *minor*.

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NOTE: Since submitting our manuscript for publication there has appeared a report by M. Girbardt (Archiv. Mikrobiol. **39**: 351–359. 1961) of an electron microscopical study of *Polystictus versicolor* L. ex Fr. His micrographs show structures associated with the hyphal walls comparable to those that we have identified as lomasomes.

THE GENUS PHAEODAEDALEA

M. E. P. Kauffmann Fidalgo [1]

(with 23 figures)

In 1913, Lloyd called attention to a *Daedalea, D. sprucei* Berk., with colored spores, which according to McGinty should be removed to a new genus, *Phaeodaedalea*. This epithet is considered as not validly published because, as has been discussed and demonstrated by Donk (**1951, 1960**), the names given by Lloyd under the "nom de plume" McGinty were not definitely acceptable to Lloyd himself, the publishing author (Art. 33, International Code of Botanical Nomenclature, 1956).

Daedalea sprucei has a highly variable hymenial surface and for this reason has been described as new species under three other genera: *Hexagona* Fr., *Lenzites* Fr. and *Irpex* Fr. The species seems to be inappropriately placed in any of the genera to which it has already been assigned. Macroscopically it looks similar to species of *Daedalea* Pers. ex Fr., *Gloeophyllum* Karst. and *Xerotus* Fr.; however anatomically, discrepancies may be observed in *D. sprucei*. The type species of *Daedalea* and of *Lenzites* possess hyaline, cylindric spores, with thin walls and hyaline skeletal hyphae (at times somewhat fuscescent in *Daedalea*) whereas *Daedalea sprucei* has globose to subglobose, brown spores, with a thickened and colored external wall; *Hexagona* and *Gloeophyllum* are typified by species with a hyphal system similar to that observed in *D. sprucei*, but with spores always hyaline, not thickened and cylindric-ellipsoid; the type species of *Xerotus* has a dimitic hyphal system, with generative and binding hyphae, very different from the trimitic hyphal system of *Daedalea sprucei;* the type species of *Irpex* has hyaline spores and hyphae.

I conclude that *Daedalea sprucei* is inappropriate to any of the polyporaceous genera referred to above and I have no alternative but to consider it as a single species of a new genus. The name *Phaeodaedalea* will be used for this new genus (Art. 72, note of the Code).

Several genera of Polyporaceae, mostly created by Murrill and Karsten, have colored spores as one of their characteristics: *Amauroderma* Murr., *Coltricia* S. F. Gray, *Coltriciella* Murr., *Cycloporus*

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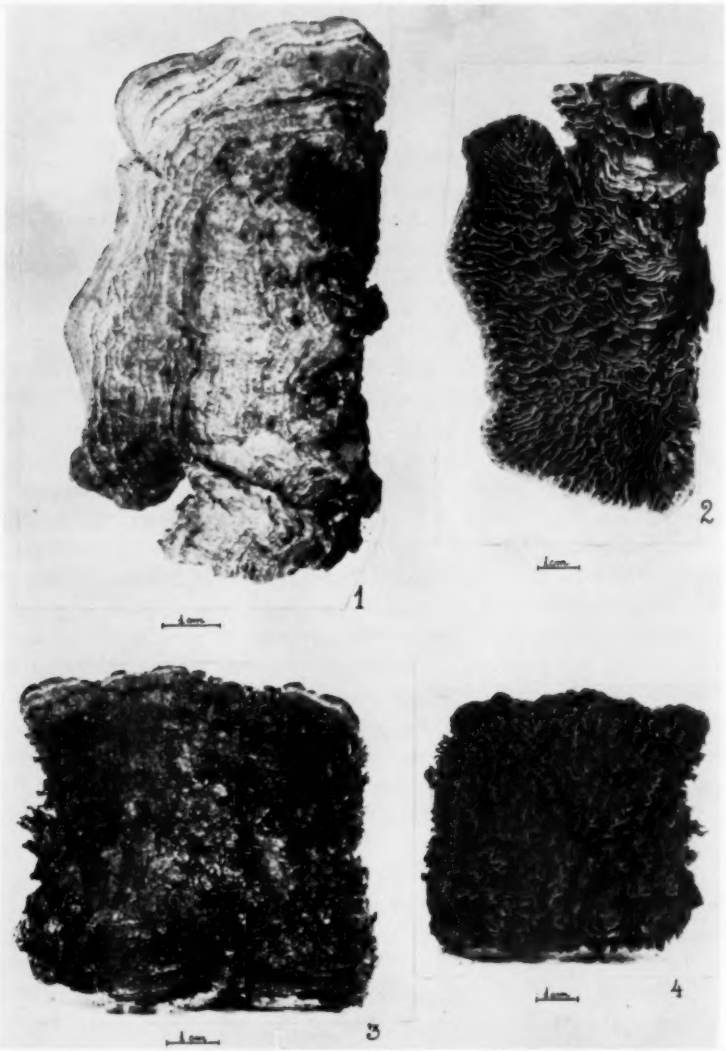
Murr., *Elfvingia* P. Karst., *Fomitiporella* Murr., *Fuscoporella* Murr., *Ganoderma* P. Karst., *Globifomes* Murr., *Inodermus* Quél., *Inonotus* P. Karst., *Melanoporella* Murr., *Melanoporia* Murr., *Phellinus* Quél.



FIGS. 1–4. *Phaeodaedalea sprucei*. 1, 2. Pilear surface and lenzitoid hymenial surface of specimen from Amazonas State, Brazil; 3, 4. Pilear surface and irpicoid-daedaloid hymenial surface of specimen from São Paulo State, Brazil.

(pro parte) and *Pyropolyporus* Murr. In studying these I found nothing closely related to *Phaeodaedalea*, which is morphologically analogous to the polyporaceous genera with hyaline spores like *Daedalea*, *Hexagona*, and *Gloeophyllum*, although in reality very distant from them having in mind the color and shape of the spores.

PHAEODAEDALEA K. Fidalgo, gen. nov.

Fructificatio sessilis, grandis, dimidiata, flabelliformis aut applanata, frequenter resupinata; suberosa; superficies pilei primo tomentosa dein glabrescens, zonata, sub-sulcata vel tuberculata; contextum homogeneum, fuscum; superficies hymenialis primo labyrinthiformis dein lamelliformis, cum alveolis hexagonibus aut irpiciformibus, vinosa dein umbrina; systema hyphale trimiticum; hyphae genitales hyalinae, septatae, fibulatae, brachiatae, non tunicatae; hyphae skeletales flavo-fuscae, aseptatae, abrachiatae, crasse tunicatae vel sub-solidae; hyphae connectantes hyalinae, aseptatae, brachiatae, sub-solidae vel solidae; basidia curta, hyalina; sporae flavo-fuscae, globosae vel sub-globosae, tunicatae et laeves; hymenium cystidiis instructum sed setis et metis destitutum. Species typica: *Daedalea sprucei* Berk.

Fruiting body sessile, dimidiate, flabelliform, applanate or resupinate, corky; pilear surface tomentose, then glabrescent, zonate, subsulcate or tuberculate; context homogeneous, medium to dark brown; hymenial surface initially daedaloid, then lamelliform, with hexagonal pores or irpiciform, vinous when fresh, fuscous brown by drying.

Hyphal system trimitic; generative hyphae hyaline, septate, with clamps; skeletal hyphae yellowish brown; binding hyphae hyaline. Spores globose to subglobose, brown, with smooth and thickened external wall. Basidia short, hyaline, subclavate, with short and roundish sterigmata. Cystidia yellowish brown, thickened, fusiform to ventricose. Setae and hyphal pegs not observed.

Phaeodaedalea sprucei (Berk.) K. Fidalgo, comb. nov. FIGS. 1–22

Daedalea sprucei Berk. in Hooker's Jour. Bot. **8**: 236. 1856; Berkeley & Cooke, Jour. Linn. Soc. (Bot.) **15**: 385. 1877; Cooke, Grevillea **10**: 133. 1882; Murrill, North Amer. Flora **9**: 125. 1908; Lloyd, Mycol. Writ. **4** (Let. 44): 9. 1913; Murrill, Mycologia **11**: 26. 1919; Wakefield, Kew Bull., Misc. Inf. **6**: 250. 1934; Rick, Broteria **33**: 159. 1937; Torrend, Ann. Reun. sulamer. Bot. **2**: 326. (1938) 1940.

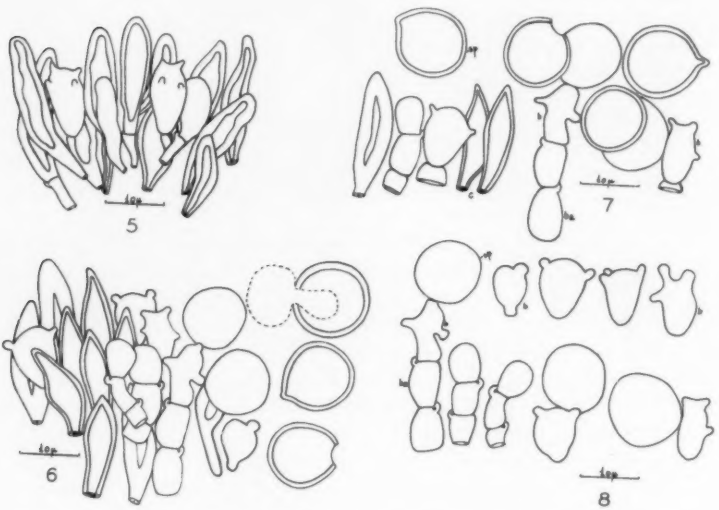
Hexagona erubescens Berk. in Hooker's Jour. Bot. **8**: 237. 1856. (TYPE from Panuré, Brazil.); Berkeley & Cooke, Jour. Linn. Soc. (Bot.) **15**: 386. 1877; Lloyd, Mycol. Writ. **3** (Syn. Hex.): 21–22. 1910; Torrend, Broteria **4**: 114. 1935.

Hexagona aequalis Pat., Jour. Bot. **3**: 258. 1889. (TYPE from

S. Carlos do Rio Negro, Venezuela.); Lloyd, Mycol. Writ. **3** (Syn. Hex.): 33–34. 1910; Torrend, Broteria **4**: 118. 1935.

Lenzites distancifolia Romell, K. svenska Vetensk. Akad. Handl. **26**: 12. 1901—reprint. (TYPE from Buritĭ, Serra da Chapada, Mato Grosso, Brazil.).

Irpex rickii Lloyd, Mycol. Writ. **7** (no. 75): 1358. 1925. (TYPE from South Brazil.); Stevenson & Cash, Bull. Lloyd Lib. **35**: 72. 1936.



FIGS. 5–8. *Phaeodaedalea sprucei.* 5. Hymenium of an old fruiting body; 6. Hymenium of fresh material collected in dry place; 7, 8. Hymenial elements. sp. = spore; b = basidium; ba = basal cell; c = cystidium.

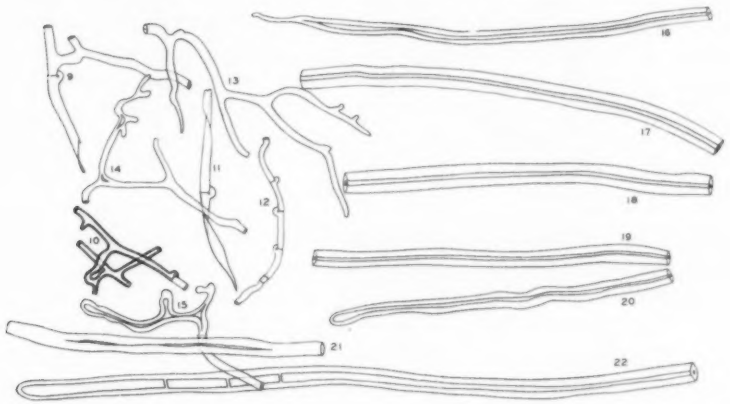
Following the recommendation 23 Bi and 45 A of the Code I have not included in the synonymy two other names not validly published. One is *Irpex sprucei* Lloyd, a herbarium name for the type of *Irpex rickii* Lloyd, which I do not intend to validate here. The other is *Phaeodaedalea sprucei,* a name for which the author, Lloyd, did not assume responsibility giving it under the "nom de plume" McGinty.

Type locality: Panuré, Amazonas, Brazil.
TYPE specimen: Kew Herbarium, collected by Spruce no. 41.

Illustrations: Patouillard, Essai taxon. Hyménomycètes 90, fig. C. 1900 (as *Hexagona aequalis*); Romell, K. svenska Vetensk. Akad. Handl. **26**:

tab. 1, fig. 13. 1901 (as *Lenzites distancifolia*); Lloyd, Mycol. Writ. **3** (Syn. Hex.): fig. 301. 1910 (as *Hexagona erubescens*); Mycol. Writ. **3** (Syn. Hex.): fig. 322. 1910 (as *Hexagona aequalis*); Mycol. Writ. **7** (no. 75): pl. 338, fig. 3212. 1925 (as *Daedalea sprucei*); Mycol. Writ. **7** (no. 75): pl. 338, fig. 3211. 1925 (as *Irpex rickii*).

Fruiting body sessile, large, dimidiate, flabelliform to applanate, often entirely resupinate, (2–)4–17(–20) × (1.5–)4–12 × (0.4–)0.7–3(–4.5) cm, corky, flexible in the thin specimens; pileus tomentose to glabrescent, zonate, subsulcate to tuberculate, dark avellaneous (Seguy 131, Brun havane, MP 14D7, Adobe or MP 15C8, Chukker brown),[2] generally of



FIGS. 9–22. Hyphae of *Phaeodaedalea sprucei*. 9–12. Generative hyphae; 13–15. Binding hyphae; 16–22. Skeletal hyphae.

a darker color behind; margin thin, acute and lighter in color than the rest of the pileus; context homogeneous, very thin, 0.1–1(–2) cm thick, medium brown (Seguy 336, Bure, MP 14E7, Stag or MP 15E9); hymenial surface initially daedaloid, with flexuous pores, then very variable, lamellate, hexagonal or irpiciform, most commonly irpiciform behind, vinous when fresh (Seguy 686, Brun de Garance, Seguy 711, MP 8E8, African + or MP 56H2), darkening when dry and changing to dark brown (Seguy 116, Bistre foncé or MP 16A12, Biskra), with age turning slightly gray (Seguy 176, Terre d'ombre brulée, Seguy 702 or MP 15C7, Racquet); pores or lamellae with very acute and rigid edges, generally undulate, crenate or fimbriate; lamellae, tubes or teeth (0.2–)1–

[2] Abbreviation and name of color nomenclature found in this paper are relative to: MP—Maerz, A. & M. R. Paul. A dictionary of color. 2nd ed. 208 p., 1–56 pl. 1950; Seguy—Seguy, E. Code universel des couleurs. Encycl. prat. Nat. 30: 1–48. pl. + 68 p. 1936.

4.5 cm deep; dissepiments (300–)400–800(–1500) μ thick; pores or lamellae in the margin, (3–)4–6(–8) per cm.

Hyphal system trimitic; generative hyphae hyaline, septate, clamped, branched, thinwalled to slightly thickened, 1.5–2.5(–3) μ diam; skeletal hyphae yellowish brown, not branched, nonseptate, thickened to subsolid, 3–4 μ diam; binding hyphae hyaline, much branched, nonseptate, subsolid to solid, 1–2.5(–3) μ diam. Cystidia fusiform to ventricose, yellow to yellowish brown, much thickened, (13–)14.5–25(–27) × (4–)5–7 μ. Basidia subclavate, short, hyaline, 1–4 sterigmate, (8–)9–10 × 5–7 μ, with sterigma very short and roundish, 1–1.5 μ long, some tetrasterigmate basidia present two apical sterigmata and two others laterally placed. Spores globose to subglobose, not amyloid, yellowish brown, slightly apiculate, thickened, 9.5–11.5(–13) × 10.5–13.5(–15) μ, with a smooth and brown outer layer, 1–1.5(–2) μ diam, not stained by the phloxin, which however stains the interior of the spore dark pink. Hyphal pegs and setae not observed. Hymenium thickening by the stratification, of the same type found in some species of *Hymenochaete*, in the old fruiting bodies reaching (80–)100–150 μ thick, presenting several layers of basidia and cystidia.

Berkeley apparently was the first to describe this species, giving it two names (*Daedalea sprucei* and *Hexagona erubescens*) simultaneously (**1856**, p. 236, 237), based on two collections found by Spruce in Amazonas State, Brazil. These two collections had different hymenial surfaces, one described as *D. sprucei* was dark-brown with daedaloid-lamellate hymenium whereas that published as *H. erubescens* had a vinous hymenium with flexuous pores. It may be concluded that Berkeley's mistake was made not only because of the variation of this species but, also because of the different ages of the two collections. The type specimen of *H. erubescens* consisted of much younger material which presented the typical vinous color of the young specimens of *Phaeodaedalea sprucei*. Bresadola (**1916**) compared the types of the two species of Berkeley and considered *H. erubescens* as a form of *D. sprucei*.

Lloyd seemed to have a very confused idea about this species. *Daedalea sprucei* was considered as the type of a new genus, *Phaeodaedalea* McGinty (a name not validly published), but all the synonyms were not included by Lloyd in the new genus. So *Hexagona erubescens* Berk. is indicated as a good species; *Hexagona aequalis* Pat. is said to be a tropical form of *Daedalea quercina* (L.) Pers. ex Fr. and an irpiciform collection of *P. sprucei* was described by Lloyd as *Irpex rickii*.

Hexagona aequalis was described by Patouillard (**1889**) as an allied species to *Hexagona glabra* Lév., which according to Bresadola is a later synonym of *Daedalea pruinosa* Lév. Lloyd (**1910**, p. 33) and Torrend

(**1935**, p. 118) considered *H. aequalis* as a tropical form of *Daedalea quercina*. The examination of the type specimen of *H. aequalis*, found in the Farlow herbarium, proved it to be a specimen of *P. sprucei*. On the label of this material there is a note, possibly from Patouillard himself, as following: "*Hexagona aequalis* Pat. = *Daedalea sprucei* Berk., n'a rien de commun avec *Daedalea quercina*." Bresadola (**1916**) included *H. aequalis* also in the synonymy of *D. sprucei*.

Cunningham (**1950**, p. 243) inserted in the synonymy of *Coriolus sprucei* (Berk.) G. Cunn. the name of *Daedalea sprucei* Berk. The first is based on *Trametes sprucei* Berk., a species described from Amazonas State, Brazil, but completely different from *D. sprucei* Berk. Cooke (**1892**) had reported these two species in Australia, *Trametes sprucei* under no. 845 and *Daedalea sprucei* under no. 873, but never with the same generic epithet.

Rick (**1937**, p. 159) had considered *Daedalea sprucei* as a good species. However in the posthumous paper (Rick, **1960**, p. 261) he did not mention it, but only *Irpex rickii* Lloyd, which he recognized as a form "resupinata irpicoidea *D. sprucei*" and added to the synonymy of *Daedalea quercina* var. *stalactiformis* Mont. Apparently this is a typographic error since the Latin diagnosis that follows the name of *D. quercina* var. *stalactiformis* does not fit, as it presents amongst other characteristics spores "pallidis, 12 μ, sphaericis."

The basidium in this species appears to have a very variable shape. In the old and dry specimens basidia are quite difficult to find, indeed very few were observed. These are subclavate, short, tetrasterigmate, with very short and round sterigmata. In fresh material, collected in São Paulo State, Brazil, basidia were more frequent, presenting subglobose to subclavate shapes, with few short cells at the base and with variable number of sterigmata, 1–4. In some tetrasterigmate basidia the sterigmata are placed in a very unusual position, two of them being apical and the other two lateral. Several of these basidia still had spores attached to the sterigma.

Specimens examined: SOUTH AMERICA. Brazil: Amazonas—Panuré, *Spruce 40* (TYPE of *Hexagona erubescens*, Herb. Bresadola, BPI[3]); Cachoeira de Tarumã, Manaus, *P. Occhioni* (RBM 382, SP 19989); Baia—locality unknown, *C. Torrend* (BPI); Mato Grosso—Buriti, *G. Malme 1029 B* (TYPE of *Lenzites distancifolia*, BPI); Rio Grande do

[3] The abbreviations of herbaria found in this paper follow the indications of the Index Herbariorum (Part I, Herbaria of the World. 1956). RBM corresponds to Mycological herbarium of Jardim Botânico do Rio de Janeiro, Guanabara, Brazil.

Sul—locality unknown, *J. Rick* (BPI); locality unknown, *J. Rick* (TYPE of *Irpex rickii*, Lloyd Coll. 24117, BPI, found in the herbarium under the name *Irpex sprucei*); São Paulo—Estrada Araraquara—São José do Rio Preto, Km 10, *J. S. Furtado* (SP 58113). British Guiana: Agatasb, Essequibo River, *D. H. Linder 811* (BPI); Kareai Creek, Essequibo River, *D. H. Linder 626* (BPI). Dutch Guiana: Paramaribo, *J. Knyper* (Lloyd Coll. 55721, BPI). Venezuela: San Carlos do Rio Negro, *L. Savoye* (TYPE of *Hexagona aequalis*, Herb. Patouillard, FH 2674). CENTRAL AMERICA. British Honduras: Temash River, *W. A. Schipp 954* (BPI).

Habitat: Few references are found in literature concerning the hosts of this species, also few notes by collectors are found in the herbaria. It is

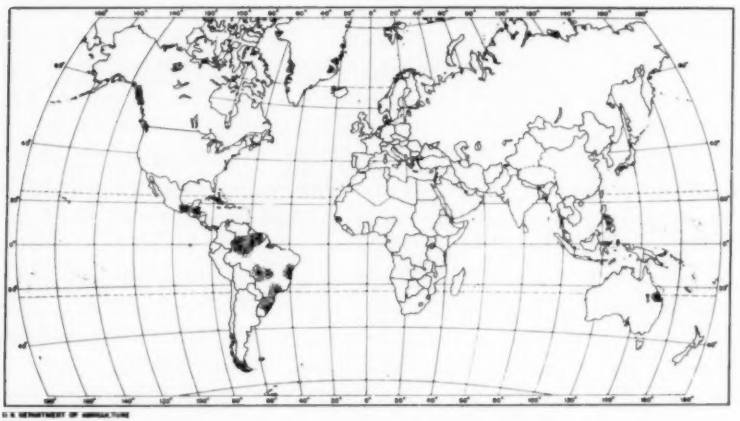


FIG. 23. Geographical distribution of *Phacodaedalea sprucei*.

reported to be found only on dead trunks, with the exception of two cases, one reported by Murrill (**1919**, p. 26) indicating it on dead and living trunks and the other by Wakefield (**1934**, p. 250) who received some material from British Guiana found on dead bark of living *Cassia pteridophylla* Sand.

Distribution: The distribution of this species is apparently limited to South America, Central America and West Indies, occurring in the tropical and temperate zones (FIG. 23). It has been reported from few places so far: Brazil, Venezuela, British and Dutch Guianas, British Honduras and according to Murrill (**1919**, p. 26 and **1912**, p. 52) also from Cuba and Mexico (Jalapa).

In Brazil this species has been collected in few states, which are located in very different and distant geographical areas, which may indicate that *P. sprucei* may be found in other Brazilian states. Up to the present it has been reported from: Amazonas, Baia, Mato Grosso, S. Paulo, Goiaz and Rio Grande do Sul.

Outside the American continent the species was reported by Cooke (**1892**, n. 873) from Queensland, Australia. It is impossible to confirm this report since no available material was found for study outside America.

SUMMARY

Phaeodaedalea gen. nov., of the family Polyporaceae, is published based on *Daedalea sprucei* Berk. This species had already been indicated by Lloyd as belonging to a new genus, but no valid description was published. The important characters are the colored and globose to subglobose spores, the trimitic hyphal system and the high variation of the basidium, with 1–4 sterigmata.

Other polyporaceous genera having colored spores were studied but none are closely related to *Phaeodaedalea,* which is morphologically analogous to the polyporaceous genera with hyaline spores such as *Daedalea* and *Gloeophyllum.*

Habitat, geographical distribution, synonymy, macroscopic and microscopic characters of *Phaeodaedalea sprucei* are presented as well as a list of the American specimens examined.

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REVIEWS

RADIATION BOTANY, a new botanical journal.

Volume 1, Number 1, of RADIATION BOTANY appeared September 1961. It is an international journal devoted to plant radiobiology and closely related topics. Dr. A. H. Sparrow, Brookhaven National Laboratory, is Editor-in-chief and the journal is published by Pergamon Press, New York. Annual subscription rates are $20.00 for libraries and $10.00 for individual subscribers. It is expected that about 400 pages will appear yearly in four issues.

Although all aspects of radiobiology of plants (including fungi) will be considered, the journal will be primarily concerned with the effects of ionizing radiation on plants or plant structures. Technical aspects of apparatus and techniques will also be included. Original contributions in English, French, or German are accepted, and brief reports, letters to the Editor, book reviews, and invited review articles will be published.

The physical appearance of the first issue is satisfactory with a double column format, adequate subheadings, and excellent reproduction of figures and plates. The board of editors is distinguished and international.

Sufficient research on the radiobiology of plants, if broadly interpreted, is currently being conducted to warrant the publication of this new journal.—RICHARD M. KLEIN.

THE BIOLOGY OF THE FUNGI, by C. T. INGOLD. 124 p, illus. Hutchinson Educational Ltd., London, 1961. Price, 12s 6d.

In his preface, Dr. Ingold reflects, "It will be a sad day when students of fungi cease to marvel at the beauty of structure in moulds and toadstools, and cease to enquire into how they function as living mechanisms." This statement is the keynote of this small book. The title is apt, since the book is not primarily concerned with any particular group of fungi but explores many corners of the complex assemblage. Dr. Ingold has long been interested in the physiological functions of fungi, especially in

spore dispersal and dispersal mechanisms. The book's preface opens with the lines, "In this little book I have tried very simply to set out my own attitude to mycology." With this aim, the reader is not disappointed, as the author skims through the fungi, here pausing to expound on a structure of interest, there ignoring whole groups in order to hurry on to another perhaps more interesting phenomenon.

Dr. Ingold claims not to be a taxonomist and does not dwell on the subject. Indeed, in some instances, he dismisses classification as out of hand. He feels that there are more important topics to be discussed, and that time cannot be wasted with systematics, when it is sex, habit or dispersal that really warrants attention. The posteriorly uniflagellate Phycomycetes and Mycetozoa are excluded from the text. Ecological groups of fungi are stressed with only the barest mention of classification. For example, the Fungi Imperfecti are divided into the Sphaeropsidales and Hyphomycetes, with no mention of either the Saccardian or Hughesian systems of taxonomy. Ascomycete and basidiomycete taxonomy are used only as tools to get to the organisms themselves.

All this would seem to require some knowledge of the fungi by the reader; such is the case throughout the text. Reference is made to the relationships between the Saccharomycetales and Endomycetales, but the latter group is not defined, nor are the relationships elaborated. The author obviously presupposes an audience of mycologists. Why then does he go to lengths to explain the principles of pH, or the rudiments of culture technique?

Dr. Ingold has the rare quality of being both concise and lucid, which leads to a very well written text. The illustrations have been gleaned from various publications and, for the most part, present the material well. Certain sections are very expressive, especially those on protoplasmic streaming in *Phycomyces* (p. 20), ascospore liberation and crozier formation in the Discomycetes (pp. 47–50), sexuality in the Basidiomycetes (pp. 77–79) and fairy ring formation (p. 84). The final chapter summarizes the primary points of ecology and parasitism.

Perhaps not quite popular enough for the layman, and too popularized to serve as a major reference work, the book would seem to have no place in the rapid-fire, impersonal gristmill of American science. However, for those who would pause to consider their work leisurely, and for those who know and respect Dr. Ingold and his experience, the book needs no recommendation. It stands on its own merit; one man's look at the fungi.—RONALD H. PETERSEN.

THE GENUS TILLETIA, by Ruben Duran and George W. Fischer. 138 p, 64 figs. Washington State University Press, Pullman, Wash. 1961. Price, $2.75.

This brief monograph of the important smut genus *Tilletia* brings together the known species of the world. Seventy-six species are described, including one new species, *T. cathcartae,* from Australia; 27 species are reported for the United States. The microscopic and macroscopic characters used in the keys are clearly described, particular attention being given to the spore wall characters. The taxonomic key is based on soral and spore characters and host symptomatology; this is followed by a host-genus key. Each species is fully described with synonymy, soral and spore descriptions, hosts, and range. There are comments on many of the species. An outstanding feature of the book is the excellent illustrations, consisting of photomicrographs of 64 of the species. The descriptions are followed by a list of excluded species (44), literature cited, a brief discussion, and an index. The authors use *Tilletia controversa,* whereas most writers, including Kühn who wrote the original description, use *T. contraversa.* The book is printed on good paper which shows the photomicrographs to advantage. This should prove to be a very useful and valuable book.—S. M. PADY.

INTRODUCTION TO SOIL MICROBIOLOGY, by Martin Alexander. x + 472 p, illus. John Wiley & Sons, Inc., New York and London, 1961. Price, $9.50.

This textbook will serve well its chosen purpose, the introduction of the microbiologist and the agronomist to the dynamic character of the soil. Each chapter is a happy combination of informal discussion and generalization, presentation of principles without drowning them in words and facts, and review of important facets of pertinent research—all supported admirably by excellent graphs, tables, and diagrams of biochemical conversion pathways. All students in this field should welcome the high degree of readability that has been enhanced by apt choice of word and turn of phrase.

Early chapters scan the major taxonomic groups of microorganisms found in the soil and generalize on their ecology, distribution and abundance, reaction to environment, taxonomy, activity, and function. The over-all excellence of illustrations is marred here by one set of line-

drawings labeled with generic names of fungi; the figures of *"Aspergillus"* and of *"Mucor"* barely surpass a doodling stage.

The major role of microorganisms in the decomposition of complex organic material is handled with emphasis on the microbiological and chemical aspects of cellulose, hemicellulose, and lignin decomposition. One chapter of special interest is devoted to biological transformation of hydrocarbons and degradation of pesticides in the soil. Several chapters cover aspects of the nitrogen cycle and of microbial transformations of phosphorus, sulfur, iron, and other minerals. A good emphasis on ecological interrelationships, maintained throughout, is summarized in two final chapters on biological equilibrium and microbiology of the rhizosphere.—EMORY G. SIMMONS.

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Established by The New York Botanical Garden in 1909, in continuation of the Journal of Mycology, founded by W. A. Kellerman, J. B. Ellis, and B. M. Everhart in 1885. Edited by William Alphonso Murrill, 1909–1924. Edited by Fred Jay Seaver, 1924–1946; by Alexander H. Smith, 1946–1950. Edited by G. W. Martin, 1951–1957. Beginning with January, 1933, the official organ of the Mycological Society of America.

North American Flora. Descriptions of the wild plants of North America, including Greenland, the West Indies, and Central America. Planned to be completed in 34 volumes. Roy. 8vo. Each volume to consist of four or more parts. [Not offered in exchange.] Volumes 1–10 devoted to fungi.

Vol. 1, part 1, 1909. Myxomycetes.

Vol. 2, part 1, 1937. Blastocladiaceae, Monoblepharidaceae, Saprolegniaceae, Ectrogellaceae, Leptomitaceae.

Vol. 3, part 1, 1910. Nectriaceae-Fimetariaceae.

Vol. 6, part 1, 1922. Phyllactiniaceae (part).

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Vol. 9 (now complete), parts 1–7, 1907–1916. Polyporaceae-Agaricaceae (part).

Vol. 10, part 1, 1914; parts 2 and 3, 1917; part 4, 1924; part 5, 1932. Agaricese (part).

Series II, part 1, 1954. Tuberales.

Brittonia, quarterly; beginning in 1957, official publication of the American Society for Plant Taxonomists. $7.50 a year.

Economic Botany, quarterly; review and research articles dealing with plants useful to man; established 1947; beginning 1959, the official journal for The Society for Economic Botany. $8.00 a year.

The Memoirs of The New York Botanical Garden, issued irregularly beginning in 1900; approximately 500 pages per volume. $10.00 a volume.

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The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. By Henry A. Gleason. 3 volumes. 1952, slightly revised 1958. List price $35.00 per set; shipping charge $1.00. Succeeds the Illustrated Flora by Nathaniel L. Britton and Addison Brown. Includes descriptions and drawings of the plant species, from ferns to orchids, which grow without cultivation in the area extending from the St. Lawrence River to Virginia and westward to Missouri and Minnesota.

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